SP1 Mouse Brain Organisation and Interspecies Comparisons – Second Data Results package (update on implementation of the DMP - M24) (D1.6.2 – SGA2)

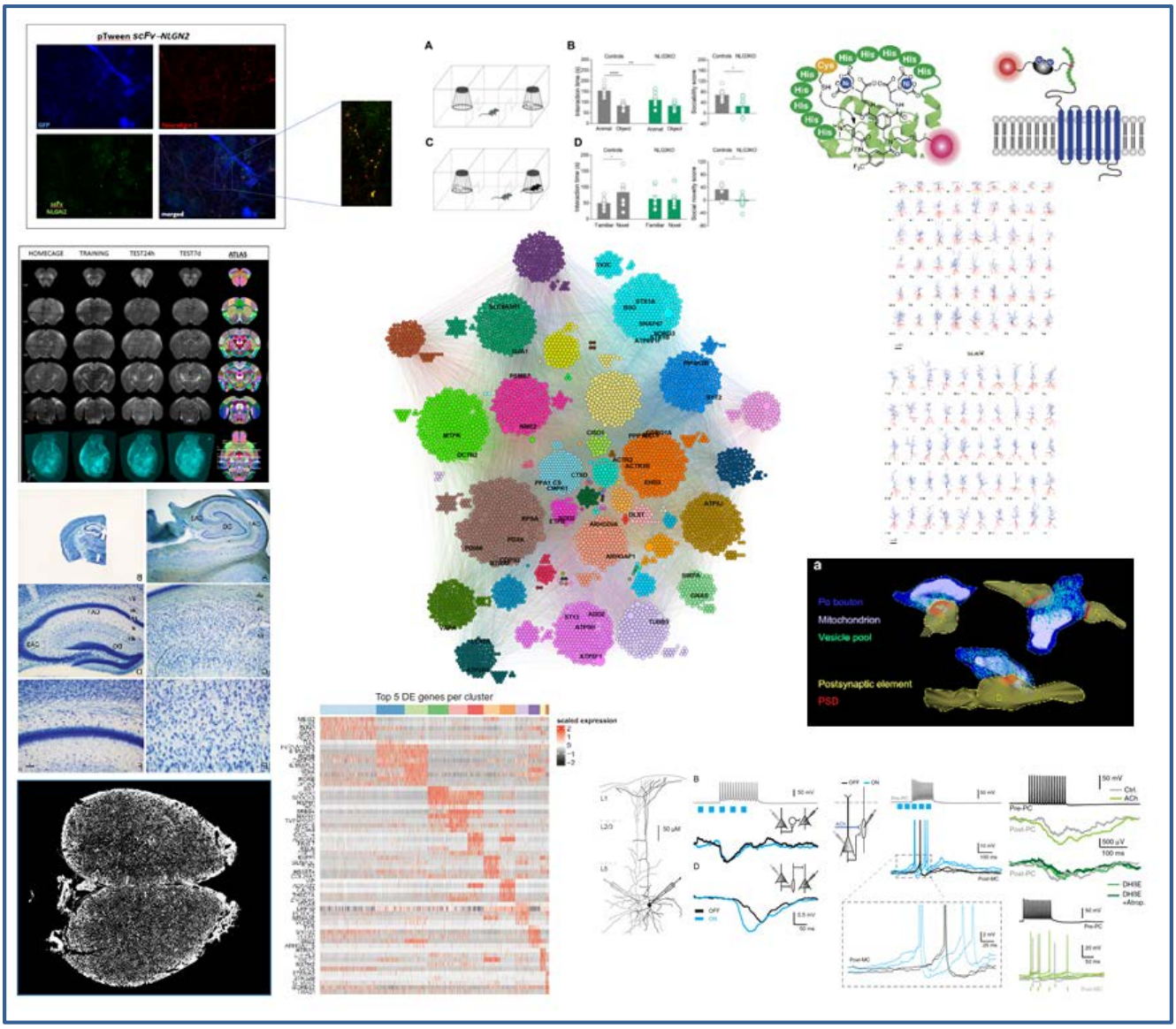

Figure 1: Examples of outputs contributing to SP1 Key Results

Examples of outputs contributing to SP1 Key Results in the period M13-M24 from different laboratories

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Description in GA:	For consistent presentation of HBP results, SGA2 M24 Deliverables describing the accomplishments of an entire SP, WP or CDP have been prepared according to a standard template, which focuses on Key Results and the outputs that contribute to them. Project management elements such as Milestones and Risks will be covered, as per normal practice, in the SGA2 Project Periodic Report.
Abstract:	This Deliverable outlines the main SP1 outputs during the period M13-M24. These outputs are main the final 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, an update on implementation of the SP Data Management Plan by M24.
Keywords:	Molecular and Subcellular, Cellular and Microcircuits, Whole-Brain, Datasets, IT tools, models, multi-level 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
25 May 2020	Deliverable submitted to EC
06 Aug 2020	Resubmission with specified changes requested in Review Report Main changes requested: • Change 1: References to components need to be added (extract from Review Report) • Change 2: References to WPs, tasks and Outputs need to be added (extract from Review Report) • Change 3: ToC containing almost only numbers (extract from Review Report) • Change 4: Full list of related publication need to be added
11 Aug 2020 10 Sep 2020	Revised draft sent by SP/CDP to PCO. Main changes made, with indication where each change was made: • Change 1: References to components included in a new annex (see Annex 1: SP1 Components) • Change 2: References to WPs, Tasks and Outputs added (see Sections 1-9 and Annex 2: SP1 Objectives and WP structure) • Change 3: ToC updated • Change 4: Full list of related publications added at the end of each section • Change 5: Links updated with the final public links or embargo lifted (Sections 3.1.5, 4.1.3, 5.1.3, 5.1.4, 6.1.6, 6.1.7, 6.2.1 and 7.1.6) • Change 6: Data embargoed stated (Sections 4.1.2, 4.1.5, 4.1.6, 5.1.3 and 5.1.4) • Change 7: Revision of Table 26 (total figures were corrected)
23 Sep 2020	Revised version resubmitted to EC by PCO via SyGMa
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1. Overview

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

During the SGA2, SP1 has focused on the whole brain and on four major brain circuits: neocortex (including the thalamocortical system), hippocampus, basal ganglia and cerebellum with the goal to examine the molecular, genetic and anatomical patterns separately in these particular regions. A major goal during SGA2 has been the integration of multilevel data. Furthermore, another important contribution of SP1 is the human-rodent comparative anatomical and physiological studies.

For the second data results package, SP1 has generated final data mainly on the mouse brain and — to a limited degree— on human brain tissue, and has achieved significant advances by generating high-level datasets at different levels for modelling proposes, by performing multi-level approaches and by implementing 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.

At the end of the second Project year, it can be considered that SP1 has met the planned objectives (SO1.1-SO1.5, see Annex 2: SP1 Objectives and WP structure) by achieving the five Key Results proposed in line with the SP1 data strategy.

This Deliverable complies with the HBP DMP and Data Policy to guarantee the quality and availability of Outputs.

2. Introduction

The overarching objective of SP1 is to generate neuroscientific concepts, knowledge, experimental datasets and tools, which will be used to build models for the simulation of the brain. In addition, SP1 will provide data and knowledge to support activities undertaken by other SPs and CDPs (mainly SP6 Brain Simulation Platform, but also SP4 Theoretical Neuroscience, SP10 Neurorobotics Platform) and CDPs (mainly CDP1 and CDP2). SP1, in agreement with the modelling pipeline of HBP, has been focused on four major brain circuits: neocortex (including the thalamocortical system), hippocampus, basal ganglia and cerebellum. A unique contribution to the categorisation and standardisation of multiscale brain data has been released by using high resolution analysis of neuron and neuronal microcircuits, both structurally and functionally. In addition, research at brain-wide scale has been performed to provide a systemic –rather than a regional view of the mouse brain and to obtain a multiscale and multilevel integration, from microcircuitry up to whole brain. These data are fundamental for validation of simulation of brain functionality and connectivity.

The SP1 work has been focused on fundamental questions, coordinated at the HBP level, on structural organisation, neuronal activity, microcircuit dynamics, synaptic plasticity and neuromodulation required to fuel and complement modelling and theory. The data have served for both model reconstruction and validation in virtuous feed-back cycles between simulations and experimental recordings (data generate models and simulations instruct hypothesis-driven data sampling). The data have also been used to obtain high quality integrative maps and circuits of the mouse brain at the functional and anatomical level, integrating SP5 databasing and brain atlasing with molecular, morphological and functional data. SP1 research has adopted all advanced techniques required for SP1 to meet the needs of in particular of the Brain Simulation Platform (SP6). This has included performing inter-domain analyses and across-scale investigations encompassing molecular, anatomical and functional data integration in rodents, and carry out comparative studies of cells and microcircuits in the rodent and human brain. This report outlines the main SP1 Outputs during the second year of SGA2 that have been published.

A total of 28 Outputs are outlined involving 53 datasets (four of them still under a patenting process and part of the data are still under embargo in the KG), 2 models, and 5 IT tools. The Outputs described in this Deliverable contribute to these KRs to meet the SP1 Objectives (SO1.1-SO1.2, SO1.3, SO1.4 and SO1.5, see Annex 2: SP1 Objectives and WP structure). High-quality molecular/subcellular full datasets have been generated for hypothesis- and data-driven brain modelling. In this regard, a major Output has been the integration of anatomical and functional studies on the four main brain regions (neocortex (including thalamus), hippocampus, basal ganglia and cerebellum. Furthermore, new datasets have been obtained on brain structure and function, and multi-scale investigations on brain physiology and long-range connectivity. Altogether, these datasets provide a valuable reference for HBP simulations at brain-wide scale. Moreover, a new framework including novel tools for integration, visualisation and analysis of anatomical and functional data has been implemented across different scales. Finally, full anatomical and functional datasets have been released as well as new statistical models have been developed to perform comparative studies across species.

Outputs outlined are described at KR level and their validation as well as their actual and potential use is also displayed. Main examples of publications generated by these Outputs are listed at the end of each KR. In addition, at the end of the report, a summary of the update on implementation of the SP1 Data Management Plan (SP1 DMP) is also included. In this summary, the contents of the second data results package are included. In addition, as the datasets generated in the second Project year complement datasets obtained in the period M1-M12, a general overview of the SP1 DMP in line with the KRs is displayed in this section. This overview includes qualitative and quantitative information of the Outputs included in this report as well as the other dataset, models and IT tools generated in this SP1 in the period M1-M24.

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

3.1 Outputs

3.1.1 Overview of Outputs

Additional to the 8 main Outputs contributed to KR1.1 (see SGA2 Deliverable D1.6.1 (D7.1 D5) for further details) by M12, at M24, 9 more Outputs have contributed to KR1.1 which aims at generating high-quality molecular, subcellular, cellular data needed for imaging, mapping, proteomics and physiology to inform brain modelling of HBP. The Outputs provide results towards the achievement of SO1.1: *Generate high-quality molecular/subcellular data needed for hypothesis- and data-driven brain modelling (mostly by SP6 Brain Simulation Platform, CDP1, and CDP2)*, and are outlined below.

3.1.1.1 List of Outputs contributing to this KR

- Output 1: Imaging of Amyloid Beta Oligomers (C1767)
- Output 2: Imaging of Neuroligin2 and Gephyrin (C1767)
- Output 3: Intrabody interference with trans-synaptic proteins: biochemical and cellular data (C1770)
- Output 4: Development of AVV vectors for SynActive tool (C1767)
- Output 5: Proteomics of potentiated spines: the PSD-95 interactome (C1770)
- Output 6: Mapping of potentiated dendritic spines (C1770)
- Output 7: Interfering with transsynaptic signalling in the hippocampus severely impairs GABAergic neurotransmission (C1770)
- Output 8: Models of Short and Long-Term synaptic plasticity including subcellular pathway (C1770)
- Output 9: Receptor and ion channel distribution in neocortex, hippocampus and cerebellum (C1887)

Details of the components contributing to this KR can be found in Annex 1: SP1 Components.

3.1.1.2 How Outputs relate to each other and the Key Result

Outputs relate to KR1.1 by generating the high-level datasets at molecular and subcellular level. Output 1, 2 and 3 generate multi-level data (molecular, subcellular and synaptic) on different molecules. Output 1, 2 and 3, are reciprocally related as achievements of the same HBP innovation, “Nanobody platform”. Output 2 is a prerequisite of Output 3. Output 3 is a prerequisite of Output 7; Output 4 provides new SynActive tool for imaging and functional information of potentiated spines. This Output is connected to Outputs 5 and 6 because all of them give subcellular information of potentiated spines and are achievements of “SynActive Platform” HBP Innovation; Output 5 provides unique data on the protein composition of potentiated spines compared to the non-potentiated ones. This Output relates to Output 4 and 6 as stated above; Output 6 gives imaging and mapping data of potentiated spines. It relates to Outputs 4 and 5; Output 7 gives new functional data on transsynaptic signalling obtained by molecular interference strategy. It relates to Output 3 for intrabody validation and 8 for being the data input; Output 8 is a developed model of synaptic plasticity at cellular and subcellular level. It is connected to Outputs 3 and 7 for the above and

mutual reasons; Output 9 relates to KR1.1 by generating subcellular data on receptors and ion channel distribution along the neuronal surface of neurons in the hippocampus, neocortex and cerebellum. The datasets are the first 2D map of receptors and ion channels.

3.1.2 Output 1: Imaging of Amyloid Beta Oligomers

Imaging of Amyloid Beta Oligomers (AbetaOs), well-known target in Alzheimer's Disease (AD), is an urgent but critical issue due to their structural complexity and difficult accessibility in human brains. To this aim, in the HBP we developed conformational anti-AbetaOs nanobodies, here exploited for an improved imaging of AbetaOs in human AD brains. In the current state of the art, our final data represent the first evidence of new patterns of AbetaOs in human brains. We report the following insights: a) antibody-based structural information on AbetaOs deposits in human brains; b) AbetaOs intracellular localisation against the prevailing hypothesis of extracellular plaque deposits; c) significant intracellular AbetaOs accumulation in pyramidal neurons of layer 3 and 5 of human cortex, with a relevant colocalisation with autophagic and lysosomal vesicles. Of note one anti-AbetaOs nanobody was recently exploited for intrabody interference *in vivo*, in brains of pre-symptomatic AD mouse model (Scopa *et al.*, 2019).

Component released: C1767. Data are not submitted to curation in KG, for patenting reasons. This Output has contributed to publication P2141 (Scopa *et al*. 2019). Dataset has not yet been not published but this Output has contributed to publication P2141 by a molecular tool that has been exploited, in a way (intrabody interference) that is different from brain imaging, which is the focus of the Output and the subject related to the patenting process. Thus, the results of the Output are not published as such in publication P2141 and they are still confidential and embargoed before publication and patenting process.

3.1.3 Output 2: Imaging of Neuroligin2 (NLG)2 and Gephyrin

NLG2 and Gephyrin are post-synaptic co-interactors, involved in trans-synaptic signalling, which play several roles in synaptic function and signal transmission, mainly via GABA-A receptors in inhibitory synapses. According to neuroscientists in HBP and outside, a reliable imaging of NLG2 and Gephyrin was considered a critical experimental issue. Hereto, we exploited the innovative Nanobody platform to select new nanobodies for an improved brain imaging, also used as intrabodies in Output 3 and 7. Here, we report new data on nanobody imaging of NLG2 and Gephyrin in mouse brain. In detail, anti-NLG2 scFv#1, validated in NLG2-KO mouse, and anti-Geph, recognise subsets of synaptic puncta in mouse brain, which are only partially detected by currently available commercial antibodies. This shows new and improved features in antigen recognition of nanobodies, making them exploitable for new mapping purposes of inhibitory synapses in mouse and human brain.

Component released: C1767. Data have not been submitted to curation in KG, for patenting reasons. This Output has contributed to publication P2359, which is a review describing Gephyrin molecular organisation and function, also mentioning intrabody interference, but not describing new data on brain imaging, which is the focus of the Output and the subject related to a patenting process. Thus, results of the Output are not published as such in publication P2359 and they are still confidential and embargoed before publication and patenting process.

3.1.4 Output 3: Intrabody interference with trans-synaptic proteins: biochemical and cellular data

Investigating trans- and post-synaptic proteins is of main interest inside and outside of the HBP but very challenging due to the complexity of their interactome. To this aim, by exploiting the Nanobody platform, we generated new intrabodies targeting different trans-synaptic proteins such as NLG2 and Gephyrin (see also Output 2), NLG3 and Neurexin NRX3β. We exploited this unique toolbox to produce multi-level data ranging from imaging (see Output 1), to functional protein interference and computational modelling (see Outputs 7 and 8). Here, we report datasets on the following intrabodies: a) two anti-NRX3β (VHH283, VHH316), unique binders for NRX3β in literature, tested by

co-IP assay; b) four new anti-NLG3 intrabodies (VHH18, VHH27, VHH36 and VHH107) raised against a NLG3-specific extracellular domain; c) two anti-NLG2 intrabodies (#1 and #645), raised against the Gephyrin Binding Domain of NLG2. Anti-NLG2 intrabodies show differential expression patterns in mouse primary cortical neurons. In particular, only anti-NLG2#645 localises in dendritic spots of endogenous NLGN2 (detected by double immunolabelling), representing the best intrabody candidate for functional interference (see Output 7). Moreover, anti-NLGN2#1 and #645 intrabodies, expressed in neuroblastoma N2A cells, determine an increase of dendritic branching. This novel evidence allows understanding new mechanisms of action of NLGN2 through its gephyrin interaction. Component released: C1770. Data not submitted to curation in KG, for patenting reasons. This Output has contributed to publication P2359 (Pizzarelli *et al*., 2019). Dataset is not published yet but this Output has contributed to the publication P2359, a review describing Gephyrin molecular organization and function, mentioning intrabody interference but not against targets described in this Output (namely NRX3B ang NLG2).

3.1.5 Output 4: Development of AVV vectors for SynActive tool

Table 1: Output 4 Links

Component	Link to	URL
C1767	Data Repository	Mapping of potentiated synapses in mouse hippocampus and organotypic hippocampal slices using SynActive constructs: DOI: https://doi.org/10.25493/TK68-40P https://kg.ebrains.eu/search/instances/Dataset/78af62dd-5d8f-4410-bc3c-30213aea599e An optogenetic probe for the specific manipulation of newly potentiated dendritic spines: DOI: https://doi.org/10.25493/5B62-88V https://kg.ebrains.eu/search/instances/Dataset/54ef2f17-2c88-48c4-99ce-1a90a2e1da58
	Technical Documentation	Data descriptor in the KG.
	User Documentation	Data descriptor in the KG.

We have created a new SynActive tool for *in vivo* optogenetic manipulation of potentiated synapses. In particular, the SynActive tool for optogenetic assays created at M18 has been inserted into a rAAV vector for *in vivo* usage. This tool is currently being evaluated via stereotaxic injection into the mouse hippocampus. It can be used to assess the necessity and the sufficiency of synaptic engrams associated to behavioural output of a specific memory in physiological and in pathological conditions, in which memory processes are altered such as Alzheimer's Disease.

3.1.6 Output 5: Proteomics of potentiated spines: the PSD-95 interactome

Table 2: Output 5 Links

Component	Link to	URL
C1770	Data Repository	DOI: https://doi.org/10.25493/H836-W4N https://kg.ebrains.eu/search/?facet_type%5B0%5D=Dataset#Dataset/6d866bc3-ddf2-4c81-9969-2b2d66be58db (embargoed)
	Technical Documentation	Data descriptor in the KG
	User Documentation	Data descriptor in the KG

We have updated our datasets of the PSD95 interactome at potentiated spines of hippocampus by performing further biological replicates and by analysing data to isolate the molecular fingerprint of potentiated dendritic spines. These data are unique because in addition to provide the background for modelling studies of synaptic plasticity at the nanoscale, they provide the first hippocampal PSD95-interactome of potentiated spines versus constitutive (not potentiated) spines. The data and results obtained so far identify for the first time the molecular fingerprint of potentiated synapses.

3.1.7 Output 6: Mapping of potentiated dendritic spines

Table 3: Output 6 Links

Component	Link to	URL
C1770	Data Repository	DOI: https://doi.org/10.25493/TK68-40P https://kg.ebrains.eu/search/?facet_type%5B0%5D=Dataset#Dataset/78af62dd-5d8f-4410-bc3c-30213aea599e (embargoed).
	Technical Documentation	Data descriptor in the KG
	User Documentation	Data descriptor in the KG

Imaging datasets of SynActive positive (potentiated) synapses obtained from i) mice which underwent memory and learning task and from ii) organotypic hippocampal slices following *ex vivo* paradigms of "synaptic engrams" have been released. Preliminary data from the analysis of potentiated spine distribution along the dendritic tree of neurons expressing the SynActive imaging tool for imaging have been released. Data and results obtained up to now provide the first evidence of the distribution of potentiated dendritic spines in neurons recruited by learning and memory processes and following *ex vivo* synaptic plasticity protocols.

3.1.8 Output 7: Interfering with transsynaptic signalling in the hippocampus severely impairs GABAergic neurotransmission

We tested the intrabody against Neuroligin 2, anti-NLG2#645, newly developed in C1767 (see Output 3), by patch-clamp recordings of spontaneous inhibitory currents from lentivirally-transduced pyramidal neurons in organotypic hippocampal slices. We found that this selective protein interference significantly hampered GABAergic synapses.

This technological advance enables a temporal and spatial control of the interference on the inhibition and gives the opportunity to specifically target protein-protein interaction edges compared to the gene-based knock-out or RNA interference approaches that target the nodes of the intracellular protein networks, and do not allow targeting selectively the edges of the protein interaction network. Component released: C1770. Data not submitted to curation in KG, for patenting reasons, This Output has contributed to publication P2388 (Modi *et al*., 2019).

3.1.9 Output 8: Models of Short and Long-Term synaptic plasticity including subcellular pathway

Table 4: Output 8 Links

Component	Link to	URL
C1770	Model Repository	https://kg.ebrains.eu/search/instances/Model/eb1825d33997ec554caf4f9ad2313b2a
	Technical Documentation	At the above link
	User Documentation	At the above link

This Output belongs to C1770 release "Models of Short and Long-Term synaptic plasticity including subcellular pathway".

Part of the data from Output 7, contribute to this Use Case. We report on a new model of the subcellular pathways for Gephyrin (GEPH) and neuroligin2/neurexin (NLG2/NRXN), including short-term plasticity effects. A new Use Case has been implemented in the Collab and the model has been integrated into the Brain Simulation Platform. Detailed information on these models can be found in SGA2 M20 Deliverable D1.1.1 (D2.1 D52).

3.1.10 Output 9: Receptor and ion channel distribution in neocortex, hippocampus and cerebellum

Table 5: Output 9 Links

Component	Link to	URL
C1887	Data Repository	DOI: https://doi.org/10.25493/XZ0C-R9G https://kg.ebrains.eu/search/instances/Dataset/1cfea99c-b9ee-4748-b900-6ed6db944435
	Technical Documentation	'Data descriptor' in the KG.
	User Documentation	'Data descriptor' in the KG.

This Output is the final release of the component receptor and ion channel distribution in neocortex, hippocampus and cerebellum (C1887). Final quality datasets have been released on neurotransmitter receptors and ion channels in the hippocampus of C57BL/J6 mice. Data are used to build the 2D map of receptor and ion channel distribution along the neuronal surface of neurons in the hippocampus, neocortex and cerebellum. The datasets are the first 2D map of receptor and ion channel. No use of the Output yet for modelling proposes. Most datasets are now curated (hippocampus) and others (cerebellum and neocortex) are in curation. This Output contributes to this KR and also to CDP2 in terms of the usage of data from the cerebellum. This Output has contributed to publication P2219 (Zenmyo *et al*., 2019) and publication P2549 (Tabata *et al*., 2019).

3.2 Validation and Impact

3.2.1 Actual and Potential Use of Output(s)

Outputs 1-2-3:

Outputs 1 and 2 generate innovative tools for imaging (nanobodies) ready to be exploitable by other groups in HBP (i.e. C1767 User Group) and already in use out of HBP (i.e. Dr. KORONYO, USA; Dr. GOBBI, Italy). An improved imaging will contribute to HBP Brain Atlases. Output 3 (already exploited by Output 7 in mouse) is exploitable *in vitro* in human cultured neurons/brain slices. Studies of patentability are in progress, i.e. for development of diagnostic (Output 1) and tools of study (Outputs 2, 3) relevant for medical or industrial bodies outside the HBP. Validation measures are Milestones achieved, MS1.1.6 "Nanobodies generation and uses for high resolution brain imaging / mapping of different antigens" (Outputs 1-2), and MS1.1.7 "Anatomical, functional and biochemical characterization of synaptic plasticity/activated spines in control and experimental models of synaptic interference" (Output 3). Outputs generate final quality data, validated by internal and external users.

Output 4: The SynActive-optogenetics tool is in the advanced validation step. Once the protocol for its *in vivo* usage will be set, it will be distributed to both internal and external users. It will be instrumental in shifting the scale for studying the substrates for synaptic plasticity from the cellular to the single-synapse level.

Output 5: The datasets have been used by C1611, C1612, and C1776 for molecular models of synapses, thus contributing to KR1.4. External user ORI A (Jena), who collaborates with SNS in the proteomic analyses of the transfected samples.

Output 6: The SynActive tool for imaging of potentiated synapses has been validated in an *in vivo* study (Gobbo *et al*. in preparation). These data can be exploited for modelling the structure of a single potentiated synapse, and for validating computational models of synaptic plasticity. The SynActive tool for imaging is currently used by the following external users: MORRIS R (UK), KELLY T (Univ of Bonn), LOSONCZY A and POLLEUX F (Columbia University), ALBERINI C (NYU Center for Neural Science), WENBIAO G (New York University), WHITING P and AGHAIZU D (UCL). Their feedback is good. A manuscript from SNS HBP partner and KELLY T is in preparation. SynActive is also applicable to *ex-vivo* human tissue. Gathering proteomic and mapping data on potentiated spines from human organotypic brain slices and neurons derived from human induced Pluripotent Stem Cells will provide unevaluable molecular and imaging data for the spatio-temporal plasticity models

Outputs 7 and 8: Since M20, HBP user MIGLIORE M (CNR) has exploited part of these datasets for modelling synaptic plasticity of hippocampus (Output 8). It will be used by SP6 to model short-term plasticity and will be included in the model catalogue as soon as the corresponding publication is online. With this model, users will be able to study the direct (and compensatory) changes in specific subcellular biochemical pathways. Validation measure of these two outputs are MS1.1.7 and SGA2 M20 Deliverable D1.1.1 (D2.1 D52).

Output 9: The datasets of receptors and channel densities are mature and ready to be used for modelling. This Output of receptor and channel densities make possible to use realistic parameters for modelling, and to visualise any membrane proteins of interest in the HBP Research Infrastructure. This Output could be also used outside the HBP to constrain models of rodents' neurons of the hippocampus and cerebellum microcircuits.

3.2.2 Publications

Publications generated from the Ouputs listed in this section are as follows:

- P2141: Impaired adult neurogenesis is an early event in Alzheimer's disease neurodegeneration, mediated by intracellular Aß oligomers Cell and Death Differentiation Scopa, C., Marrocco, F., Latina, V., Ruggeri, F., *et al*, 2019, DOI 10.1038/s41418-019-0409-3

  This publication confirms that Outputs 1, 3 and 7 have been validated by scientific peer review. The publication: (i) shows an innovative application *in vivo* of the scFvA13 anti-AbetaOs nanobody, tool described in Output 1; and (ii) covers the validation of lentiviral expression system for intrabodies used in primary cultures in Output 3 and in organotypic slices in Output 7.

- P2359: Tuning GABAergic Inhibition: Gephyrin Molecular Organization and Functions. Pizzarelli, R., Griguoli, M., Zacchi, P. *et al*., DOI: 10.1016/j.neuroscience.2019.07.036.

  Outputs 2 and 8 have been validated by scientific peer review via this publication. The publication reviews the role of Gephyrin in GABAergic transmission, showing the relevance of intrabody approach targeting Gephyrin.

- P2388: Possible Implication of the CA2 Hippocampal Circuit in Social Cognition Deficits Observed in the Neuroligin 3 Knock-Out Mouse, a Non-Syndromic Animal Model of Autism. Modi, B., Pimpinella, D., Pazienti, A., Zacchi, P., Cherubini, E., and Griguol, M. 2019. http://dx.doi.org/10.3389/fpsyt.2019.00513 .

  This publication confirms that Output 7 has been validated by scientific peer review. This study is focused on Autism spectrum disorders (ASDs) and its results suggest that the selective alterations in network dynamics and GABAergic signalling observed in the CA2 hippocampal region of NLG3 knock-out mice may account for deficits in social memory reminiscent of those observed in autistic patients.

- P2219: Optimized Reaction Pair of the CysHis Tag and Ni(II)-NTA Probe for Highly Selective Chemical Labeling of Membrane Proteins Naoki Zenmyo, Hiroki Tokumaru, Shohei Uchinomiya,

Hirokazu Fuchida, Shigekazu Tabata, Itaru Hamachi, Ryuichi Shigemoto, Akio Ojida (2019) Bulletin of the Chemical Society of Japan, Vol. 92, No. 5**.** http://dx.doi.org/10.1246/bcsj.20190034.

This publication confirms that Output 9 has been validated by scientific peer review. This study is focused on an optimised method to develop double chemical labelling for co-localisation of receptors and ion channels in freeze-fracture replicas, a second reaction pair was developed by means of SP1 activity

- P2549: *Electron Microscopic Detection of Single Membrane Proteins by a Specific Chemical Labeling* Shigekazu Tabata, Marijo Jevtic, Nobutaka Kurashige, Hirokazu Fuchida, Munetsugu Kido, Kazushi Tani, Naoki Zenmyo, Shohei Uchinomiya, Harumi Harada, Makoto Itakura, Itaru Hamachi, Ryuichi Shigemoto, Akio Ojida (2019) *iScience, Vol. 22***,** http://dx.doi.org/10.1016/j.isci.2019.11.025

  This publication confirms that Output 9 has been validated by scientific peer review. A new method is implemented to develop quantitative freeze-fracture replica labelling with higher resolution and sensitivity for 2D localisation of receptors and ion channels, a new specific chemical labelling method has been established by means of SP1 activity.

4. Key Result KR1.2: High-level multiscale 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

In addition to the eight main Outputs contributing to this KR in the first Project year (see SGA2 M12 Deliverable D1.6.1 (D7.1 D5) for further details), nine more Outputs are contributing to achieve KR1.2. This KR involves the generation of high-level multiscale datasets at cellular and microcircuit level on selected brain regions: neocortex (including thalamus), hippocampus, basal ganglia and cerebellum to meet objective SO1.2: *Generate high-quality cellular and microcircuit level data needed for hypothesis and data-driven brain modelling (mostly by SP6, CDP1, and CDP2). A main focus is to integrate anatomical and functional studies on the four main brain regions: neocortex (including thalamus), hippocampus, basal ganglia and cerebellum*. Outputs of the second data results package contributing to this KR, and already published in the KG, are outlined below.

In addition to these Outputs, final datasets on dendritic architecture and axon initial segments of striatal and cerebellar neurons (C1743, C1744), on morphological and electrophysiological characterisation of hippocampal neurons (C1796, C1799), hippocampal circuitry (1795), and on GABAergic subtypes of the neocortex, have been released by M24. These datasets are currently in curation (see section 8 for further information).

4.1.1.1 List of Outputs contributing to this KR

- Output 1: 3D reconstruction, measurements and registration of cortical areal maps of the individual morphology of thalamic projection neurons form motors and sensory relay thalamic nuclei (C1867)
- Output 2: Quantitative ultrastructural analysis of the synapses established by identified thalamocortical axons in specific cortical layers (C1867)

- Output 3: Morphological and electrophysiological characterisation of striatal interneurons (C1774, C1775, C1836)
- Output 4: The micro-connectivity of the cerebellar glomerulus (C1771)
- Output 5: Detailed reconstruction of inhibitory interneurons of the cerebellar cortex (C1772)

Details of the components contributing to this KR can be found in Annex 1: SP1 Components.

4.1.1.2 How Outputs relate to each other and the Key Result

Outputs listed above are related to this KR by providing new cellular and microcircuit level data needed for hypothesis and data-driven brain modelling and the integration of the anatomical and functional studies. In particular, new datasets have been generated (i) to study the structure and function of thalamic projection neurons in the neocortex, (ii) to study neocortical microcircuits, and (iii) to characterise the structure and function of striatal interneurons.

In addition, these Outputs contribute to KR1.4 and KR1.5 as they provide key biological constrains for brain circuit modelling. Finally, these Outputs are of direct interest to KR1.3 for brain-wide neuronal circuit mapping and modelling.

Outputs are related to each other, as they contribute to achieve this KR and the objective SO1.2 consequently.

4.1.2 Output 1: 3D reconstruction, measurements and registration of cortical areal maps of the individual morphology of thalamic projection neurons form motors and sensory relay thalamic nuclei

Table 6: Output 4 Links

Component	Link to	URL
C1867	Data Repository	DOI: https://doi.org/10.25493/AWS5-MZG https://kg.ebrains.eu/search/instances/Dataset/6dee616062025ddf5fd6341ee2b865ad (embargoed)
	Technical Documentation	'Data descriptor' in the KG
	User Documentation	'Data descriptor' in the KG

This dataset describes quantitatively the somatodendritic and axonal structure of individual thalamocortical projection neurons in the thalamic somatic and visual sensory nuclei. The cells in the dataset were labeled using state-of the art viral vectors able to drive massive levels of expression of marker proteins in a neuron after a single transfection event, leaving the rest of the brain unlabelled. This allows the unambiguous visualisation /measurement of the entire axonal tree, no matter how large or complex. They are the first visualisations of these cells ever reported in mice. Experiments were conducted in vivo in wildtype C57BL76 adult animals and have a low (<5%) success rate. Accurate labelling analysis is extremely labor-intensive, especially for large and widespread-axon cells. The present dataset may thus be viewed as a collection of "archetypal" reference specimens of these neuron populations.

This Output has contributed to the publications P1961 (Casas.Torremocha *et al.*, 2019) and P2372 (Rodríguez-Moreno *et al.*, 2020).

4.1.3 Output 2: Quantitative ultrastructural analysis of the synapses established by identified thalamocortical axons in specific cortical layers

Table 7: Output 5 Links

Component	Link to	URL
C1866	Data Repository	DOI: https://doi.org/10.25493/P8XR-F6F https://kg.ebrains.eu/search/instances/Dataset/30cc895d-36c1-4b14-9cca-068df75be83d
	Technical Documentation	'Data descriptor' in the KG
	User Documentation	'Data descriptor' in the KG

Different parameters from the synapses established by thalamocortical axons coming from two thalamic nuclei (ventral posteromedial and posterior nuclei) in the somatosensory and motor cortex have been analysed and quantified. This dataset contains pictures from two types of ultrastructural analysis: serial transmission and focused ion beam milling scanning electron microscopy. Thalamocortical axons analysed in the dataset were labelled with ionthophoretic injections of biotinylated 10 K dextran amine (anterograde tracer) in a small number of neurons and revealed with the glucose oxidase-diaminobenzidine method. Experiments have been conducted in vivo in wildtype C57BL/6 adult animals. This Output has contributed to the publications P1961 (Casas.Torremocha et al., 2019) and P2372 (Rodríguez-Moreno et al., 2020).

4.1.4 Output 3: Morphological and electrophysiological characterisation of striatal interneurons

Table 8: Output 7 Links

Component	Link to	URL
C1774 C1775 C1836	Data Repository	• SPN morpho reconstructions: DOI: https://doi.org/10.25493/yp8n-hd2 • SPN ephys recordings: DOI: https://doi.org/10.25493/MZE0-BH5 • FS morph reconstructions: DOI: https://doi.org/10.25493/3FNQ-5KG • FS ephys recordings: DOI: https://doi.org/10.25493/E883-NFA • LTS morph reconstructions: DOI: https://doi.org/10.25493/DVPH-RDE • LTS ephys recordings: DOI: https://doi.org/10.25493/5GE0-6MF • ChIN morph reconstructions: DOI: https://doi.org/10.25493/ADRK-VJP • ChIN ephys recordings: DOI: https://doi.org/10.25493/3NTS-Q0B • SYN ephys recordings: DOI: https://doi.org/10.25493/487V-4AQ • ChIN morph reconstructions (2): DOI: https://doi.org/10.25493/3EV4-TDG • ChIN ephys recordings (2): DOI: https://doi.org/10.25493/VW70-659

	Technical Documentation	'Data descriptor' in the KG
	User Documentation	'Data descriptor' in the KG

This Output has contributed importantly to this KR with regard to the basal ganglia and in particular the striatal interneurons (ChIN, FSN, LTS) with morphology, connectivity and membrane properties that are being used in the simulations at SP6. In particular, different neuronal subtypes in the basal ganglia (striatum) were described. A further great detailed characterisation of the cholinergic interneurons is ready and the process of analysing the LTS (Low Threshold Striatal) interneurons has been completed. The membrane properties of the different neuron subclasses, the expressed ion channels and the detailed morphology were described in detail. In addition, 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. Released components are C1774 (morphological data), C1775 (electrophysiological data) and C1836 (electrophysiological data-SYN).

Data were released by M18 as candidate final quality data as planned. All experimental datasets are already published in the KG. Released data have contributed to publications P2075 (Suzuki *et al.*, 2019) as well as to P2076 (Grillner, S and ElManira, A., 2019 - *in validation process*), P1763 (Diederich *et al.*, 2019), P1401 (Jalalvand *et al.*, 2018), P2489 (Johannes *et al.*, 2020 *in press – SGA3 publication*), and P2465 (Suryanarayana *et al.*, 2020).

4.1.5 Output 4: The micro-connectivity of the cerebellar glomerulus

Table 9: Output 8 Links

Component	Link to	URL
C1771	Data Repository	Loose cell attached granules: https://doi.org/10.25493/6R48-E3V Voltage clamp GrC: https://doi.org/10.25493/MDAR-XEB and https://doi.org/10.25493/CHJG-7QC (embargoed) Firing Recordings of cerebellar neuronal firing induced by currents steps: https://doi.org/10.25493/4AF6-WSD and https://doi.org/10.25493/JQH3-0A4 Postsynaptic currents: https://doi.org/10.25493/F2VK-MB4 and https://doi.org/10.25493/G07Q-K87
	Technical Documentation	'Data descriptor' in the KG
	User Documentation	'Data descriptor' in the KG

We investigated the functional activation of Golgi cells along with the convergence of granule cell and Golgi cell axons in order to develop appropriate hypotheses and models of the cerebellar granular layer and molecular layer and to improve SP6 models. Electrophysiological experiments using multiple patch-clamp recordings have been used to complement histological results reported in literature. A first set of electrophysiological recordings were stored into CSCS container and another set has been released by M24 (Granule cells (28), Golgi cells (10), HD-MEA *in vitro* (5), MEA *in vivo* (4). The related morphologies are reported in C1744 and C1743 (Task T1.2.1: High-resolution reconstruction of striatal and cerebellar neurons: dendritic arbors and axon initial segments). This Output has contributed to the publications P1751 (Soda *et al.*, 2019), P1657 (Savini *et al.*, 2019), P2308 (Prestori *et al.*, 2019), P2318 (Lorenzi *et al*, 2020) P1878 (Masoli *et al.*, 2020, *in press* during SGA2, published during SGA3), and P2346 (Locatelli *et al.*, 2019).

4.1.6 Output 5: Detailed reconstruction of inhibitory interneurons of the cerebellar cortex

Table 10: Output 9 Links

Component	Link to	URL
C1772	Data Repository	Stellate cell: https://kg.ebrains.eu/search/instances/Dataset/3ca4af33-64bd-437a-9c53-2dac19e10168 DOI: 10.25493/M1AQ-3AC (embargoed) Basket cell: https://kg.ebrains.eu/search/instances/Dataset/37c082e4-02cf-407d-829a-6fbf0cffc110 DOI: 10.25493/M1V0-WE3 (embargoed) Granule cell: https://kg.ebrains.eu/search/instances/Dataset/7dc5d5d5-4323-41d6-bdfd-0b841cfe7000 DOI: 10.25493/CHJG-7QC (embargoed) Golgi cell: https://kg.ebrains.eu/search/instances/Dataset/17196b79-04db-4ea4-bb69-d20aab6f1d62 DOI: 10.25493/JNFA-HDP (embargoed)
	Technical Documentation	'Data descriptor' in the KG
	User Documentation	'Data descriptor' in the KG

A detailed reconstruction of morphologies of molecular layer interneurons (MLIs, stellate cells and basket cells) has been done to determine the spread and overlapping of axonal plexuses and develop appropriate hypotheses and models of the cerebellar granular layer and molecular layer and to improve SP6 models. Electrophysiological experiments using patch-clamp recordings and optogenetics have been used to investigate the structure-function relationship and the physiological implications of the results. The datasets are already in a CSCS container or under curation (80% completed) and will be in the container before the end of SGA2. A first set of electrophysiological recordings were stored into CSCS container and another set has been released by M24 (Granule cells (28), Stellate cells (17), Basket cells (5), Golgi cells (10), HD-MEA *in vitro* (5), MEA in vivo (4). The related morphologies are reported in C1744 and C1743 (Task T1.2.1: High-resolution reconstruction of striatal and cerebellar neurons: dendritic arbors and axon initial segments). This Output has contributed to the publications P1751 (Soda *et al*., 2019), P1657 (Savini *et al*., 2019), P2308 (Prestori *et al*., 2019), P2318 (Lorenzi *et al*, 2020), P1878 (Masoli *et al*., 2020, *in press* during SGA2, published during SGA3), and P2346 (Locatelli *et al*., 2019).

4.2 Validation and Impact

4.2.1 Actual and Potential Use of Output(s)

Outputs 1 and 2: Data on thalamo-reticular single-neuron reconstructions are being used for a model of a model of reciprocal thalamo-reticular circuits (E. LAVARONE, S. HILL, H. MARKRAM EPFL, Switzerland); The whole-brain axon constructions are being used (i) as reference data for developing automated 3D-stitching and software tools registration to digital brain atlases in a newly awarded (2019) Flag ERA Project Neurons Reunited (leader P. TIENSINGA, Donders Institute SKU) and SP5, and (ii) as key reference data for the refinement and validation of 3D virtual reality software tools (3DVR) for the reconstruction and measurement of axonal arborizations form whole-brain volumetric fMOST datasets containing labeled neurons. (Leader Dr. Hanchuan PENG, Allen Institute-Southeastern University, Nanjing, China); Finally, neuronal reconstructions are being used as key reference data for the refinement and validation of accuracy of algorhythms for the supervised-automated reconstruction of complete axonal morphologies from volumetric 3D STP datasets (Dr. Karel SVOBODA & Jayaram CHANDRASHEKAR, Janelia Fams Research Campus, NC, USA).

Output 3: These data are complementary to those released by M12 and 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. As from the beginning of the Project phase, there has been 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.

Outputs 4-5: The results related to output 4 have been published on BioRxiv (the preprint server for biology, https://doi.org/10.1101/638247) and the paper is in revision. The results related to Output 4 and 5 have been published on BioRxiv (the preprint server for biology, https://doi.org/10.1101/2019.12.19.882944) and the paper entitled "Calcium channel-dependent induction of long-term synaptic plasticity at excitatory Golgi cell synapses of cerebellum" is in revision. These Outputs have been used to implement the cerebellar models into the Brain Simulation Platform.

4.2.2 Publications

Outputs related to KR1.2 have contributed to the generation of the publications listed below.

- P2372: Area-specific synapse structure in branched posterior nucleus axons reveals a new level of complexity in thalamocortical networks. Rodriguez-Moreno J, Porrero C, Rollenhagen A, Rubio-Teves M, Casas-Torremocha D, Alonso-Nanclares L, Yakoubi R, Santuy A, Merchan-Pérez A, DeFelipe J, Lübke JHR, Clasca F., Journal of Neuroscience, 2886-19. DOI: https://doi.org/10.1523/JNEUROSCI.2886-19.2020.

  Outputs 1 and 2 have been validated by scientific peer review via this publication - This study demonstrates significant differences in the size of the boutons made in each area by individual Posterior thalamic nucleus (Po) cell axons, as well as in functionally-relevant parameters in the composition of their synapses. In addition, it was found similarly large differences between the synapses of Po versus ventral posteromedial thalamic nucleus axons in the whisker sensory cortex. Area-specific synapse structure in individual axons implies a new, unsuspected level of complexity in long-distance brain connections. This study is critical for models involving the thalamo-cortical system.

- P1961: Posterior thalamic nucleus axon terminals have different structure and functional impact in the motor and somatosensory vibrissal cortices Diana Casas-Torremocha, César Porrero, Javier Rodriguez-Moreno, María García-Amado, Joachim H. R. Lübke, Ángel Núñez, Francisco Clascá (2019) Brain Structure and Function, Vol. 224, No. 4. http://dx.doi.org/10.1007/s00429-019-01862-4

  Outputs 1 and 2 have been validated by scientific peer review via this publication. In this paper, we combined *in vivo* electrophysiology, pharmacological manipulation and optical quantitative microscopy to discover unexpected major differences in the effect of thalamocortical axon synaptic input on the neurons of the two separate cerebral cortex regions that in mice jointly control exploratory whisker movements. The decision to examine and compare these two areas was based in the discoveries we made in SP1 regarding the selective branching patterns of thalamocortical axons.

- P2075: The role of the optic tectum for visually evoked orienting and evasive movements Daichi G. Suzuki, Juan Pérez-Fernández, Tobias Wibble, Andreas A. Kardamakis, Sten Grillner (2019) Proceedings of the National Academy of Sciences, Vol. 116, No. 30, http://dx.doi.org/10.1073/pnas.1907962116.

  Output 3 has been validated by this scientific peer review via this publication. This study investigates the mechanisms underlying the selection between approaching and evasive movements. It shows that 2 classes of brainstem-projecting neurons in the deep layer of tectum, in interaction with local inhibitory interneurons, are responsible for the selective switch between

visually triggered approaching or evasive movements. This study suggests that these visual decision-making circuits are phylogenetically conserved throughout vertebrate evolution.

- P2076: Current Principles of Motor Control, with Special Reference to Vertebrate Locomotion Sten Grillner, Abdeljabbar El Manira (2019) Physiological Reviews, In Validation Process in the Deliverable submission, now validated http://dx.doi.org/10.1152/physrev.00015.2019

  Output 3 has been validated by scientific peer review via this publication. This study summarises the logics of the neural control of motion extending from the basal ganglia mechanisms responsible for selection of behaviour and cortex for precision walking to the cellular and molecular design of the central pattern generator networks in the brain stem-spinal cord. This review discusses the importance of the use of molecular markers as a complement of the morphological and electrophysiological studies to identify classes of interneurons, which are essential for generating the motor pattern and map their connectivity.

- P1401: Cerebrospinal Fluid-Contacting Neurons Sense pH Changes and Motion in the Hypothalamus Elham Jalalvand, Brita Robertson, Hervé Tostivint, Peter Löw, Peter Wallén, Sten Grillner (2018) The Journal of Neuroscience, Vol. 38, No. 35. http://dx.doi.org/10.1523/jneurosci.3359-17.2018

  Output 3 has been validated by this scientific peer review via this publication. The goal of this paper is to investigate the functional role of the somatostatin-/GABA-expressing subpopulation of CSF-c neurons, as well as to define the electrophysiological and morphological aspects of these cells. This publication reports that the somatostatin-/GABA-expressing CSF-c neurons in the lamprey hypothalamus sense bidirectional deviations in the extracellular pH and do so via different molecular mechanisms. This is important to better understanding the lamprey striatal circuits because the striatum have a rich somatostatin innervation which originates from CSF-c neurons in hypothalamus

- P2489: The microcircuits of striatum in silico J. J. Johannes Hjorth, Alexander Kozlov, Ilaria Carannante, Johanna Frost Nylén, Robert Lindroos, Yvonne Johansson, Anna Tokarska, Matthijs C. Dorst, Shreyas M. Suryanarayana, Gilad Silberberg, Jeanette Hellgren Kotaleski, Sten Grillner (2020) Proceedings of the National Academy of Sciences. http://dx.doi.org/10.1073/pnas.2000671117 (*in press* during SGA2, published during SGA3)

  Output 3 has been validated by scientific peer review via this publication. The aim of this publication is to present a nearly full-scale model of the mouse striatum using available data on synaptic connectivity, cellular morphology, and electrophysiological properties to create a microcircuit mimicking the real network. This model platform will be used to generate new hypotheses on striatal function or network dynamic phenomena.

- P1878: Parameter tuning differentiates granule cell subtypes enriching the repertoire of retransmission properties at the cerebellum input stage, Stefano Masoli, Marialuisa Tognolina, Umberto Laforenza, Francesco Moccia, Egidio D'Angelo bioRxiv 638247; doi: https://doi.org/10.1101/638247 (*in press* during SGA2, published during SGA3).

  This publication confirms that Outputs 4 and 5 have been validated by scientific peer review. This study shows that the cerebellar granule cells (GrCs) generate diverse response patterns to current injection and synaptic activation, ranging from adaptation to acceleration of firing.

- P1751: Hyper-excitability and hyper-plasticity disrupt cerebellar signal transfer in the IB2 KO mouse model of autism Teresa Soda, Lisa Mapelli, Francesca Locatelli, Laura Botta, Mitchell Goldfarb, Francesca Prestori, Egidio D'Angelo (2019) The Journal of Neuroscience. http://dx.doi.org/10.1523/jneurosci.1985-18.2019

  This publication confirms that Outputs 4 and 5 have been validated by scientific peer review. This study shows for the first time that hyperexcitability and hyperplasticity disrupt signal transfer in the granular layer of IB2 KO mice, supporting cerebellar involvement in the pathogenesis of autism spectrum disorders (ASDs).

- P1657: Default Mode Network Structural Integrity and Cerebellar Connectivity Predict Information Processing Speed Deficit in Multiple Sclerosis Giovanni Savini, Matteo Pardini, Gloria Castellazzi, Alessandro Lascialfari, Declan Chard, Egidio D'Angelo, Claudia A. M. Gandini

Wheeler-Kingshott (2019) Frontiers in Cellular Neuroscience, Vol. 13. http://dx.doi.org/10.3389/fncel.2019.00021

This publication confirms that Outputs 4 and 5 have been validated by scientific peer review. The cerebellar connectivity rules obtained in this study are fundamental for modelling the cerebellar network circuit and microcircuits.

- P2308: Diverse Neuron Properties and Complex Network Dynamics in the Cerebellar Cortical Inhibitory Circuit Francesca Prestori, Lisa Mapelli, Egidio D'Angelo (2019) Frontiers in Molecular Neuroscience, Vol. 12. http://dx.doi.org/10.3389/fnmol.2019.00267

  This publication confirms that Outputs 4 and 5 have been validated by scientific peer review. This paper summarises neuron properties and inhibitory network dynamics fundamental for single cell and microcircuit modelling.

- P2318: Lorenzi RB, Palesi F, Castellazzi G, Vitali P, Anzalone N, Bernini S, Cotta Ramusino M, Sinforiani E, Micieli G, Costa A, D'Angelo E and Gandini Wheeler-Kingshott1 CAM. Unsuspected Involvement of Spinal Cord in Alzheimer Disease, Front. Cell. Neurosci., 30 January 2020. https://doi.org/10.3389/fncel.2020.00006

  This publication confirms that Outputs 4 and 5 have been validated by scientific peer review. This work is pioneering the investigation of spinal cord alterations in patients with dementia, and in particular with AD, opening also mechanistic questions for future studies

- P2346: Calcium channel-dependent induction of long-term synaptic plasticity at excitatory Golgi cell synapses of cerebellum. F. Locatelli, T. Soda, I. Montagna, S. Tritto, L. Botta, F. Prestori, E. D'Angelo, https://doi.org/10.1101/2019.12.19.882944.

  Outputs 4 and 5 have been validated by scientific peer review via this publication- This study shows for the first time a novel form of Ca2+ channel-dependent synaptic plasticity at the excitatory synapses impinging on cerebellar Golgi cells. These results, along with recent computational predictions, support the idea that Golgi cell plasticity could play a crucial role in controlling information flow through the granular layer along with cerebellar learning and memory.

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 the SP1. More specifically, we focus on two main outputs that address brain structure and function. Outputs published in the KG are described below.

In addition to these Outputs, final datasets on Sub-cortical recording and manipulation of neuronal activity in awake mice (C2303, C2304, C2305) and on the vascular system (Brain Vascular Network Reconstruction (C1732), have been released as planned and are currently in curation.

5.1.1.1 List of Outputs contributing to this KR

- Output 1: Whole-brain datasets at sub-cellular resolution (C1798, C1797, C1761)
- Output 2: Functional connectivity of cortical neurons on GCaMP6f mice (C1765, C2304, C2391)
- Output 3: Sub-cortical recording and manipulation of neuronal activity in awake mice (C2303, C2304, C2305)

Details of the components contributing to this KR can be found in Annex 1: SP1 Components.

5.1.1.2 How Outputs relate to each other and the Key Result

The Outputs of KR1.3 are complementary and independent from each other. They are related to the KR1.3 by generating structural and functional datasets on a brain-wide scale by using cutting-edge imaging technologies.

5.1.2 Output 1: Whole brain datasets at sub-cellular resolution

Table 11: Output 1 Links

Component	Link to	URL
C1797	Data Repository, Technical Documentation and User Documentation	Whole-brain images of different neuronal markers: DOI: 10.25493/A0XN-XC1 https://kg.ebrains.eu/search/instances/Dataset/ebfe2271-8868-4cd9-8ecd-f293e3a95bd9 Documentation in 'Data descriptors' at the KG link
C1798	Data Repository, Technical Documentation and User Documentation	Whole brain images of neuronal activation: DOI: 10.25493/G20B-5QY https://kg.ebrains.eu/search/instances/Dataset/7174d02c-e9cb-40a3-874a-2ba81c47e656 (embargoed) Documentation in 'Data descriptors' at the KG link
C1761	Software Repository, Technical Documentation and User Documentation	https://github.com/lens-biophotonics/ZetaStitcher

Additional to datasets generated in the first period of SGA2, new whole-brain datasets of inhibitory interneurons have been generated, together with activation datasets (via *c-fos* labelling) under different behavioural conditions (Figure 2). Further, whole-brain vasculature datasets have been generated. Quality Control by visual inspection has been performed on each dataset by at least 2 researchers of the same laboratory. Datasets have been curated with DOIs https://doi.org/10.25493/G20B-5QY and https://doi.org/10.25493/A0XN-XC1.

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. 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.

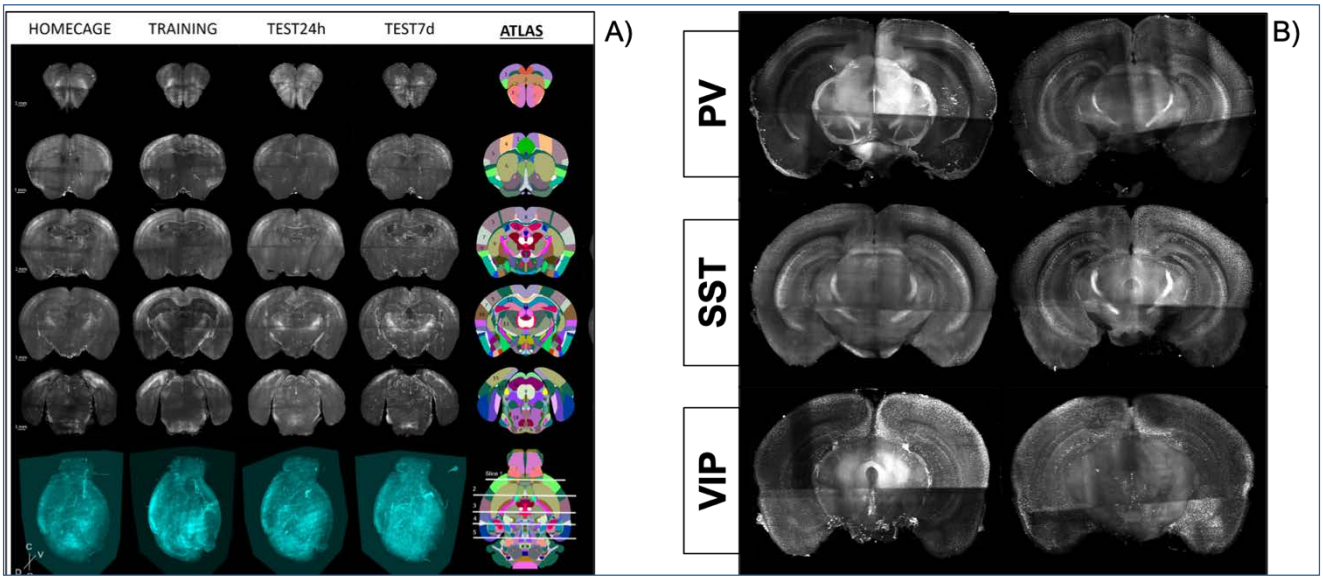

Figure 2: Whole brain datasets at subcellular resolution

A) Whole-brain neuronal activation in different phases of aversive memory observing cells expressing the immediate early gene *c-fos* (step-through behavioural paradigm of inhibitory avoidance). TRAP mice (Guenthner *et al.*, Neuron 2013) were used to label c-fos-expressing neurons. B) Distribution of the major interneuron types in the rodent brain: Parvalbumin (PV), somatostatin (SST), and vasoactive-intestinal peptide (VIP).

5.1.3 Output 2: Functional connectivity of cortical neurons on GCaMP6f mice

Table 12: Output 2 Links

Component	Link to	URL
C1765, C2304, C2391	Data Repository, Technical Documentation and User Documentation	• Resting state dataset (2P and WF): DOI: https://doi.org/10.25493/R7J6-S69 https://kg.ebrains.eu/search/instances/Dataset/3d5c5cc6-a937-42be-a630-b6d2ce657a04 (embargoed) DOI: https://doi.org/10.25493/XJR8-QCA https://kg.ebrains.eu/search/instances/Dataset/cc72a888-4c93-464d-8a48-405d8beeee5e (embargoed) DOI: https://doi.org/10.25493/3E6Y-E8G https://kg.ebrains.eu/search/instances/Dataset/71285966-8381-48f7-bd4d-f7a66afa9d79 Documentation in 'Data descriptors' in the KG links • Pulling dataset (WF): DOI: https://doi.org/10.25493/6864-QVG https://kg.ebrains.eu/search/instances/Dataset/d84060d0-c8f4-4570-8e2f-98d6e6eb4515 DOI: https://doi.org/10.25493/Z9J0-ZZQ https://kg.ebrains.eu/search/instances/Dataset/4730b8c4ae603587a04b4c4362869f91 DOI: https://doi.org/10.25493/K5HN-TFE https://kg.ebrains.eu/search/instances/Dataset/e4355d7d-d357-48cc-ab34-27f29ec7c8f3 Documentation in 'Data descriptors' in the KG links • Grasping dataset (WF): DOI: https://doi.org/10.25493/C8P7-6X0

		https://kg.ebrains.eu/search/instances/Dataset/8b7de843-2a4e-47dc-a71f-3477aeb74f34 (embargoed)

In SGA2, we extended the library of imaging datasets in anaesthetised and awake mice with wide-field and high-resolution (two-photon) imaging. We acquired new datasets with wide-field and high-resolution (two-photon) microscopy in anaesthetised mice under different anaesthesia levels and using different anaesthetics. 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. A new publication was released recently on the analysis and propagation of calcium activity (Celotto *et al*., 2020 - P2327). The combination of genetic targeting of activity indicators and fluorescence microscopy can provide highly detailed information on the contribution of specific neuronal populations (excitatory in our case) to a specific brain state and, importantly, to the transitions between states.

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 patterns 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 (Figure 3). 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.

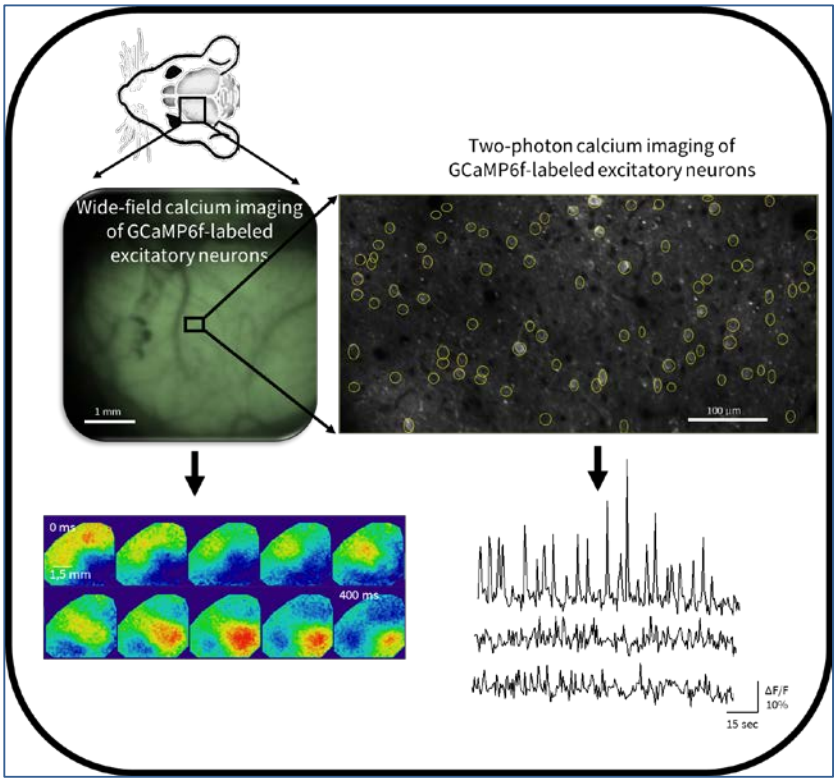

Figure 3: Multi-scale fluorescence imaging *in vivo*

To complement these data, we explored the awake brain state in mice engaged in a skilled movement. Briefly, the same mouse model was imaged with wide-field microscopy during the performance of a reach-to-grasp task. The results are reported on biorxiv (Quarta *et al*., 2020, P2516, *in validation process*). Finally, we acquired new datasets on mice before and after stroke during forelimb retraction on a robotic device. Results on related research were recently published on Cell Reports (Allegra Mascaro *et al*. (2019)- P2108).

In Table 12 the links to data repository and data description are shown. These data are two-photon and wide-field recordings of calcium activity over wide regions of the cortex (mm^2): in resting state (3 subjects TPM, 3 WF) and during voluntary movement, i.e. pulling (8 subjects WF). In addition, grasping datasets (2 subjects WF), have also been released.

5.1.4 Output 3: Sub-cortical recording and manipulation of neuronal activity in awake mice

Table 13: Output 3 Links

Component	Link to	URL
C2303, C2304, C2305	Data Repository	DOI: https://doi.org/10.25493/NK4R-2RE https://kg.ebrains.eu/search/instances/Dataset/326c3bee-ef81-4fa0-91b4-8c9cf1f17961 (embargoed)
	Technical Documentation	'Data descriptor' in the KG
	User Documentation	'Data descriptor' in the KG

In order to explore the functional connectivity of subcortical regions, we took advantage of recently developed optrodes that allow the illumination of targeted regions over a wide range of depths (Pisanello *et al*. 2017). These devices are additionally equipped with an electrode recording site along the fibre tip for simultaneous electrophysiological recordings. In DRD1-cre mice from The Jackson Laboratory (JAX, US https://www.jax.org/strain/024860), we induced the expression of the light-inducible actuator ChR2 along the entire striatum. Following optrode implantation into the striatum, these neurons were then activated by targeting the light to selected striatal regions. Thanks to an electrode recording site along the shaft of the fibre, electrophysiological recordings of dorsal striatum neurons are performed simultaneously with light stimulation. In parallel, cortical neurons of motor associated regions were genetically labelled GCaMP6f via AAV injection. The activity of these neurons associated with calcium release can thus be visualised using fluorescence microscopy. With this innovative combination of techniques, we studied the effective connectivity of striato-cortical projections in awake mice (Figure 4).

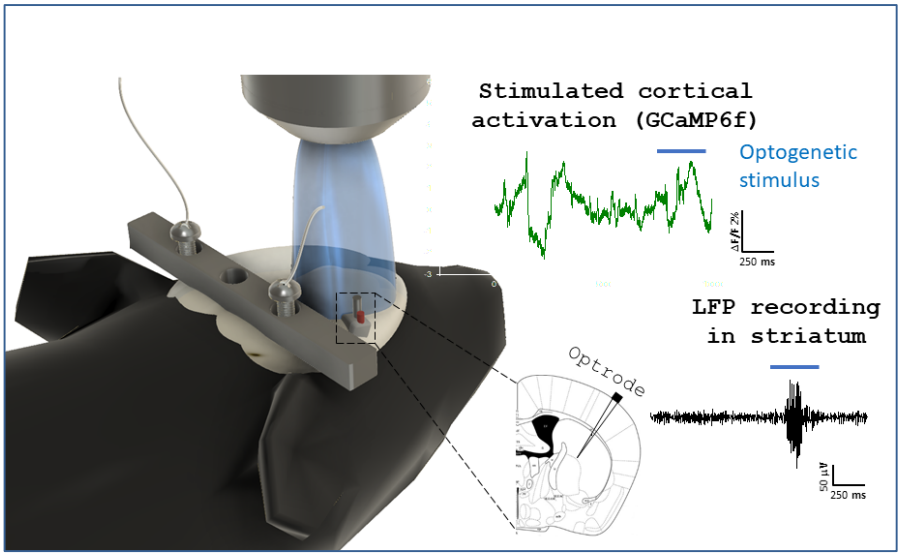

Figure 4: Simultaneous optical stimulation of selected striatal domains, LFP recordings and wide-field fluorescence imaging of cortical neurons *in vivo*

The dataset released refers to wide field microscopy images of neuronal activation in motor regions of the right hemisphere elicited by optogenetic stimulation of selected striatal regions as well as simultaneous electrophysiological recording in dorsal striatum is performed via an electrode recording site along the tapered fibre of the optrode.

5.2 Validation and Impact

5.2.1 Actual and Potential Use of Output(s)

Output 1: Since 2018, whole-brain images are used by FUA lab (EPFL, T5.3.6: Integrating Feature Extractors and Classifiers for Neuroscience) and KRESHUK lab (UHEI, then EMBL, T5.6.4: Analytics Workflow for Large-Scale Volumetric Data) in the HBP to test and develop analysis pipelines. 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 neuronal 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 imaging data have been used by SP3 and SP4 collaborators (PAOLUCCI at INFN, T3.2.5: High-efficiency multi-scale software pipeline for analysis and simulation of experimental slow waves and wakefulness transition and DESTEXHE at CNRS, T4.1.4: Biophysical models of brain signals and T4.4.1: Models of spontaneous activity and sleep) to validate theoretical models.

In detail, our SP3 collaborators (PAOLUCCI, DE BONIS, INFN) developed a data analysis pipeline 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 are described in (Celotto *et al*. (2020) - P2327). In SP4, fluorescence signals are modelled from calcium concentration fluctuations and eventually from spiking activity. In addition, SP4 is working on modelling calcium activity under different anaesthesia levels. 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. 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: Mouse Brain Function from Structure) to validate their mean-field models on whole brain activity before and after stroke (Allegra Mascaro, Falotico, Petkoski *et al*., under review). 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: Comparing activity dynamics of models and living brains), JIRSA (AMU, T4.5.2) and LASCHI (SSSA, T10.1.1: Virtual body). 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 (Adam *et al*., under review).

Output 3: Data on simultaneous subcortical and cortical recordings with optogenetic stimulation are still being recorded and are shared with our collaborators in IIT (Lecce), outside of the HBP, for validating the new optrode devices. Researchers interested in striato-cortical effective connectivity can exploit these data for validation of theoretical models.

5.2.2 Publications

Publications generated from the Ouputs listed in this section are as follows:

- P1387: Whole-Brain Vasculature Reconstruction at the Single Capillary Level. 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. Scientific reports, 2018, 8(1), 12573. http://doi:10.1038/s41598-018-30533-3 .

This publication confirms that Output 1 has been validated by scientific peer review- This publication presents a novel approach that improves vascular demarcation by combining CLARITY with a vascular staining approach that can fill the entire blood vessel lumen and imaging with light-sheet fluorescence microscopy. Furthermore, this novel method is compatible with endogenous fluorescence, thus allowing simultaneous investigations of vasculature and genetically targeted neurons. This new method will be valuable for future brain-wide investigations of the capillary network.

- P2108: Combined Rehabilitation Promotes the Recovery of Structural and Functional Features of Healthy Neuronal Networks after Stroke Anna Letizia Allegra Mascaro, Emilia Conti, Stefano Lai, Antonino Paolo Di Giovanna, Cristina Spalletti, Claudia Alia, Alessandro Panarese, Alessandro Scaglione, Leonardo Sacconi, Silvestro Micera, Matteo Caleo, Francesco Saverio Pavone (2019) Cell Reports, Vol. 28, No. 13, http://dx.doi.org/10.1016/j.celrep.2019.08.062

  Output 2 has been validated by scientific peer review via this publication. In this study, advanced optical imaging and manipulation tools are used to study cortical remodelling induced by this rehabilitation paradigm. Results provide evidence that combined rehabilitation promotes the restoration of structural and functional features distinctive of healthy neuronal networks.

- P2516: A distributed neocortical action map associated with reach-to-grasp. Eros Quarta, Alessandro Scaglione, Jessica Lucchesi, Leonardo Sacconi, Anna Letizia Allegra Mascaro, Francesco Saverio Pavone. 2020, http://dx.doi.org/10.1101/2020.01.20.911412.

  Output 2 has been validated by scientific peer review via this publication. This study shows a novel view on the neocortical correlates of motor control, with potential implications for neural repair. Data could be used to build and validate brain and spinal cord models.

- P2142: Optogenetics in Brain Research: From a Strategy to Investigate Physiological Function to a Therapeutic Tool Elena Montagni, Francesco Resta, Anna Letizia Allegra Mascaro, Francesco Saverio Pavone (2019) Photonics, Vol. 6, No. 3, http://dx.doi.org/10.3390/photonics6030092.

  This publication confirms that Sub-cortical recording and manipulation of neuronal activity in awake mice (Output 3) has been validated by scientific peer review. This publication describes the fundamental components of optogenetics, from light-activated proteins to light delivery systems. Also, it shows its applications to study neuronal circuits in physiological or pathological conditions at the cortical and subcortical level, in vivo. In addition, the interesting findings achieved using optogenetics as a therapeutic and rehabilitative tool highlight the potential of this technique for understanding and treating neurological diseases in pre-clinical models. Finally, this study shows encouraging results recently obtained by applying optogenetics in human neuronal cells *in vitro.*

6. Key Result KR1.4: Multi-level 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*. In the second project year, eight more main Outputs have been achieved to contribute to KR1.4 to meet this Objective, together with the seven main Outputs achieved in the period M1-M12 (see SGA2 M12

Deliverable D1.6.1 (D7.1 D5) for further details). These Outputs 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 were implemented across different scales. Outputs already published are described below.

6.1.1.1 List of Outputs contributing to this KR

- Output 1: Final quantitative ultrastructural data of the mouse neocortex (C1749)
- Output 2: Final quantitative anatomical data of the mouse cortical column at light microscopic level (C1748)
- Output 4: Integrated mouse and human synaptic proteome dataset with complete literature/public database (C1611)
- Output 5: Molecular rule-based model interactions and parameters pertinent to synaptic plasticity (merged component C1776 & C1777)
- Output 6: Rule-based modelling incorporated in Brain Simulation Platform (C1612)
- Output 7: Integrated framework for the interactive exploratory analysis of neurological data (C1869, C1870)
- Output 8: The basis of granule cell structural plasticity (C1773)
- Others: Output 5 from KR1.1 Proteomics of potentiated spines: the PSD-95 interactome (C1770); Outputs 1, 4 and 5 from KR1.2, Single-cell 3D reconstruction and measurement of thalamocortical ventral lateral, ventral anterior and parafascicular nuclei (C1867), The micro-connectivity of the cerebellar glomerulus (C1771) and Detailed reconstruction of inhibitory interneurons of the cerebellar cortex (C1772).

Details of the components contributing to this KR can be found in Annex 1: SP1 Components.

6.1.1.2 How Outputs relate to each other and the Key Result

Outputs relate to this KR by generating multilevel datasets by integrating anatomical, functional, and molecular data. Specifically, multilevel datasets were generated by integrating data from Outputs 1, 2 and 3 (see SGA2 M20 Deliverable D1.4.1 (D5.1 D55) for further details). Outputs 4, 5 and 6 relate to each other for the molecular integration. Output 7 relates to Outputs 1 and 2 for the developing of the IT tools to perform multilevel approaches. Finally, Output 8 relates to this KR by implementing a multilevel approach by using Outputs 8 and 9 from KR1.2.

6.1.2 Output 1: Final quantitative ultrastructural data of the mouse neocortex

Table 14: Output 1 Links

Component	Link to	URL
C1749	Data Repository	DOI: https://doi.org/10.25493/GHBT-SVN KG link: https://kg.ebrains.eu/search/instances/Dataset/5a53361c-aefa-4676-8fab-a272c53b381d
	Technical Documentation	'Data descriptor' in the KG
	User Documentation	'Data descriptor' in the KG

The final release of component C1749 has been completed and the data can be considered final quality data. The dataset comprises ultrastructural data from 35 FIB/SEM samples acquired from the six layers of the somatosensory cortex. The dataset provides densities of synapses in the different

cortical layers, distribution on different postsynaptic targets and proportions of excitatory and inhibitory synapses.

Data has been published in the KG by M24 as planned. Datasets are under embargo until publication.

6.1.3 Output 2: Final quantitative anatomical data of the mouse cortical column at light microscopic level

Table 15: Output 2 Links

Component	Link to	URL
C1748	Data Repository	DOI: https://doi.org/10.25493/QJRZ-C2X https://kg.ebrains.eu/search/instances/Dataset/ae523e54-b46a-4e01-8365-0db9d420cc1a
	Technical Documentation	'Data descriptor' in the KG
	User Documentation	'Data descriptor' in the KG

The final release of component C1748 has been completed and the data can be considered final quality data. Data on volume fraction of different cortical components of the mouse HLS1 cortical column per layer at light microscopy have been released. They have been determined in mouse (n=4) HLS1 cortical region, in 6-8 semi-thin sections (32 sections), using light microscopy and Cavalieri's principle estimations in the following cortical layers: I, II, III, IV, VA, VB and VI. In addition, the density of catecholaminergic fibres in the different layers of the mouse HLS1 has been estimated by confocal microscopy. Five TH immunocytochemically stained sections have been analysed from 3 animals.

Data have been published in the KG by M24 as planned. Datasets are under embargo until publication.

6.1.4 Output 3: Integrated Mouse and human synaptic proteome dataset with complete literature/public database

Table 16: Output 4 Links

Component	Link to	URL
C1611	Data Repository	https://kg.ebrains.eu/search/instances/Dataset/f9590f64-b8f9-4d70-a966-7af3b60ea2ae DOI: https://doi.org/10.25493/QQ55-63T
	Technical Documentation	Data descriptor in the KG
	User Documentation	Data descriptor in the KG

Synaptic function and plasticity depends on a large number of proteins on both sides of the synapse, and their interactions. It is therefore crucial to have an accurate description of what proteins are present and what their interactions are.

We have integrated systematically 57 published synaptic proteome studies, which contribute to a total of 7,617 unique mouse and human genes in the synaptic proteome. Of the 57 studies, 27 are for the postsynaptic compartment, 17 are presynaptic and 11 consider the whole synaptosome. The combined data contain 5,560 postsynaptic genes (3,439 found in two or more studies make up the "consensus" postsynaptic proteome), 2,315 presynaptic genes, and 6,888 genes reported for the synaptosome. We retrieved Protein-protein interactions (PPIs) for the list of genes to build a complete up-to-date PPI map for the whole synapse and its subcellular compartments. We embedded the datasets above in a SQLite database to enable queries based on the species, subcellular

compartment, brain region or method of identification. Our database incorporates interactions with Prof CATTANEO's (SNS, Italy) group (C1770), and they have used our database.

The database is already published in the KG (see table above).

6.1.5 Output 4: Molecular rule-based model interactions and parameters pertinent to synaptic plasticity

Table 17: Output 5 Links

Component	Link to	URL
C1776, C1777 (merged component)	Data Repository	https://github.com/oksankas/PPI_DB/blob/master/django-app/djangonautic/db.sqlite3 (data) https://kg.ebrains.eu/search/instances/Dataset/3b10cce0-c452-4217-94b4-631fff56d854 DOI: https://doi.org/10.25493/R3N8-3C1
	Technical Documentation	https://github.com/oksankas/PPI_DB and Data descriptor in the KG
	User Documentation	https://github.com/oksankas/PPI_DB and Data descriptor in the KG

Typical proteins have multiple sites at which they bind to other proteins, molecules and ions. It follows that large complexes of proteins can form, which are challenging to describe in computational models. Rule-based model languages and simulators (such as Kappa, KaSim and SpatialKappa) solve the problem of representing complexes of proteins, and allow large models to be constructed easily by combining smaller models. Each molecule or ion is called an "agent" and has named binding sites. Rules specify which sites on which agents can bind, and at what kinetic rate. In addition, biologically important modifications to sites such as phosphorylation can be described.

Our goal was to construct a number of these smaller models and make them accessible. We identified 66 molecules and ions with known contributions to synaptic plasticity processes. These include specific proteins along with protein families, making a total of 115 unique proteins. For each of the 66 molecules and ions we retrieved the domain structure and domain-domain interactions to convert the protein-protein interactions into agent and rule descriptions in the rule-based (Kappa) language. This resulted in total of 197 rules with kinetic constant information, where available, which can be used for dynamic simulation of synaptic protein complexes.

The agent and rule information is available via SQLite database (https://github.com/oksankas/PPI_DB), which could be downloaded from: https://github.com/oksankas/PPI_DB/blob/master/django-app/djangonautic/db.sqlite3, along with djiango application for user friendly interface, if required: https://github.com/oksankas/PPI_DB/tree/master/django-app/djangonautic/djangonautic.

The respective kinetic information is presented in the table: https://github.com/oksankas/PPI_DB/blob/master/Rectified%20Rules/rule_baseV0.2_fixed.xlsx

6.1.6 Output 5: Rule-based modelling incorporated in Brain Simulation Platform

Table 18: Output 6 Links

Component	Link to	URL
C1612	Software Repository	https://www.github.com/davidcsterratt/KappaNEURON
	Technical Documentation	http://dx.doi.org/10.1007/978-3-319-27656-4_9
	User Documentation	https://www.github.com/davidcsterratt/KappaNEURON

The KappaNEURON open source software allows computational modellers to incorporate detailed information about molecular interactions in synapses in the Kappa rule-based format (see Output 2/C1776/C1777) into detailed models of electrical neurons. This will help us to understand how changes in synaptic strength, which underlie learning and memory, arise out of the intricate interplay between molecular and electrical activity, and may help us to understand how disease affects synapses. KappaNEURON integrates the NEURON software package and the SpatialKappa Kappa simulator.

In SGA2 KappaNEURON was used in a student research project, which we are submitting for publication. This use leads to improvements in the software. KappaNEURON was also incorporated as an online use case in the Brain Simulation Platform (BSP), and was documented in the BSP handbook.

6.1.7 Output 6: Integrated framework for the interactive exploratory analysis of neurological data

Table 19: Output 7 Links

Component	Link to	URL
C1870	Software Repository	Vishnu: http://gmrv.es/gmrvvis/vishnu/ and https://kg.ebrains.eu/search/instances/Software/d06345f2-5032-492e-aec3-09733fac63b0 Neuronize v2: http://gmrv.es/gmrvvis/neuronizev2/ and https://kg.ebrains.eu/search/instances/Software/e99d0018-28bc-42ff-b380-abd49590eb70
	Technical Documentation	At the above links
	User Documentation	At the above links

This Output is divided in two toolsets: an integrated environment for interactive visual analysis (Vishnu), and an application designed to improve the data acquisition workflow, providing interoperability among well-established applications in the field (Neuronize v2).

The Vishnu toolset facilitates analysis operations over different sources of data, providing storage, filtering and processing of data and certain interaction between the data analysis applications that are supported. Vishnu currently supports different inputs of data, being able to store and process data from different species, individuals, populations and types (anatomical/functional) coming from various sources: CSV, Matlab, EspINA and Blue Brain's Blueconfig formats (see Figure 5). Once stored in the database and processed, the data can be inspected, selected and mixed by the neuroscientist to, later, be loaded into different data analysis tools (previously developed within Task T1.4.4: Integrated framework for the multiscale analysis of morphological and physiological data), greatly facilitating and improving the workflow of comparative studies and/or analysis.

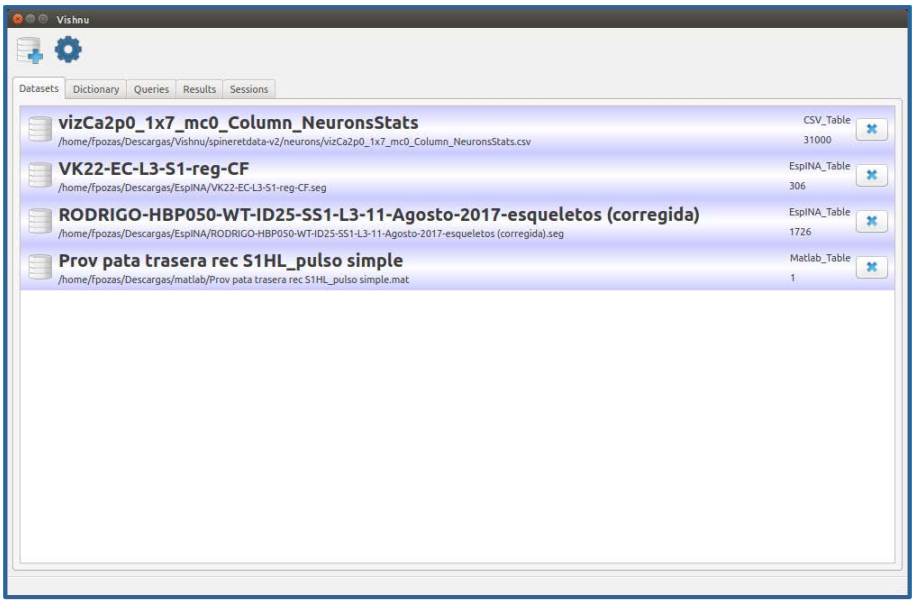

Figure 5: Vishnu allows storing data from several sources

In Vishnu, the following formats are currently supported: CSV, Matlab, EspINA and Blue Brain's Blueconfig.

Given the different origin and types of data sources, not all sources are completely processed (i.e. functional temporal series), but all are stored and available to be retrieved later in user data queries (see Figure 6). Data sources from same origin are processed together to avoid repetition of properties. For anatomical data if the shrinkage factors are provided, they can be applied to transform the data prior to the morphological analysis.

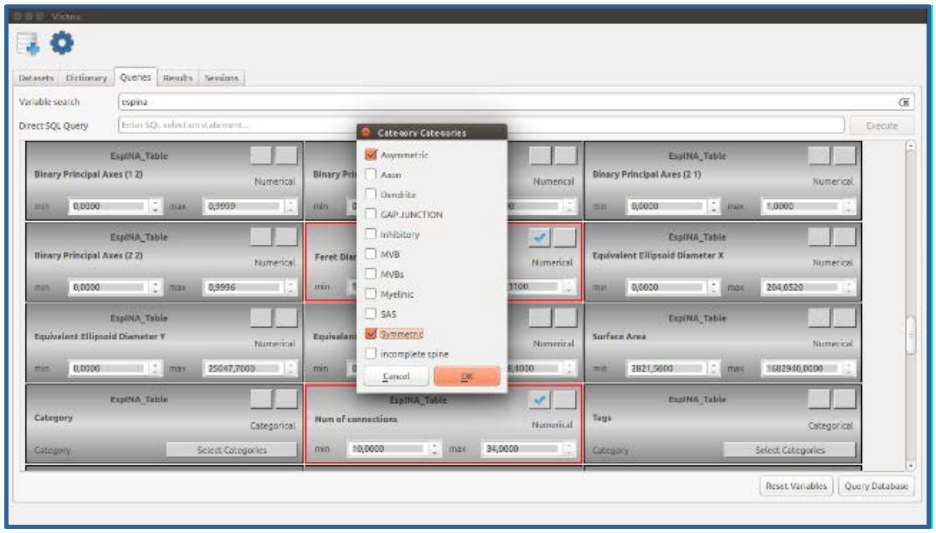

Figure 6: Users' queries are applied to all available datasets

On the other hand, Neuronize v2 resumes the work that has been done to provide interoperability between different neuroscientific tools. Neuronize v2, has been developed to enable interoperability between two of the most used software morphological tools: Neurolucida and Imaris for Neuroscientists (including its plug-in Filament Tracer). Neuronize v2 includes the possibility to take advantage of the data extracted with Imaris Filament Tracer to automatically generate a tracing with spine information that can be read directly by Neurolucida. Using this tracing, the tool also creates a 3D mesh of the whole neuron, including the spines meshes extracted from Imaris (see Figure 7).

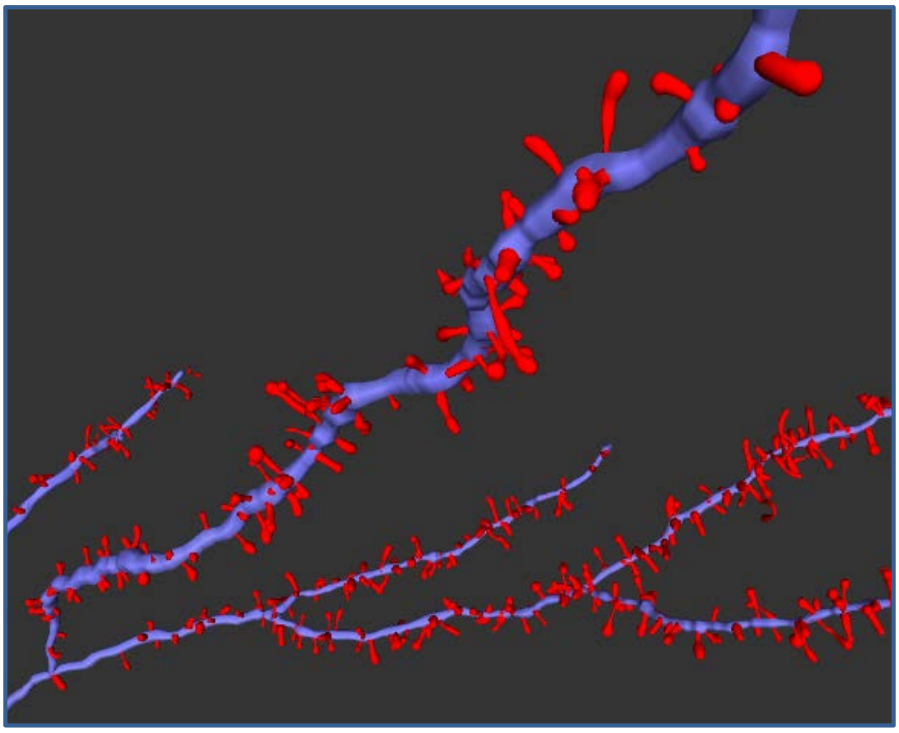

Figure 7: Imaris illustration

The image shows the spines extracted from the Imaris files placed on a mesh constructed from the neuron generated tracing.

Both, Vishnu and Neuronize v2, can be downloaded from its repositories (see the links above) and have also been uploaded in the HBP Software Catalogue.

6.1.8 Output 7: The basis of granule cell structural plasticity

Table 20: Output 8 Links

Component	Link to	URL
C1773	Data Repository	DOI: https://doi.org/10.25493/MDAR-XEB https://kg.ebrains.eu/search/instances/Dataset/40a8ed8caae4989506d69fd290b67a90
	Technical Documentation	'Data descriptor' in the KG
	User Documentation	'Data descriptor' in the KG

A detailed reconstruction of morphologies of molecular layer interneurons (MLIs, stellate cells and basket cells) has been done to determine the spread and overlapping of axonal plexuses and develop appropriate hypotheses and models of the cerebellar granular layer and molecular layer and to improve SP6 models. Electrophysiological experiments using patch-clamp recordings and optogenetics have been used to investigate the structure-function relationship and the physiological implications of the results. The datasets are already published. A first set of electrophysiological recordings has been stored into a CSCS container and another set has been published by M24: Granule cells (28) DOI:10.25493/CHJG-7QC, Stellate cells (17) DOI: 10.25493/M1AQ-3AC, Basket cells (5) DOI: 10.25493/M1V0-WE3, Golgi cells (10) DOI: 10.25493/JNFA-HDP, HD-MEA *in vitro* (5): DOI: 10.25493/4AF6-WSD, MEA *in vivo* (4) DOI: 10.25493/WEJK-XVH. The related morphologies are reported in C1744 and C1743 (Task T1.2.1: High-resolution reconstruction of striatal and cerebellar neurons: dendritic arbors and axon initial segments).

6.1.9 Others

- Output 5 from KR1.1, *Proteomics of potentiated spines: the PSD-95 interactome*, also contributes to this KR. See Section 3.1 for further details.
- Outputs 1, 4 and 5 from KR1.2, Single-cell 3D reconstruction and measurement of thalamocortical ventral lateral, ventral anterior and parafascicular nuclei, *The micro-connectivity of the cerebellar glomerulus* and *Detailed reconstruction of inhibitory interneurons of the cerebellar cortex*, also contribute to this KR. See Section 4.1 for further details.
- Outputs 1 and 3 from KR1.5, *Final datasets of the human neuropil at the ultrastructural level* and *Final quantitative ultrastructural data of the mouse hippocampus,* also contribute to this KR**.**

6.2 Validation and Impact

6.2.1 Actual and Potential Use of Output(s)

Outputs 1 and 2: Final quality data generated in this Output have been integrated along with the physiological data from Output 2 (see SGA2 M20 Deliverable D1.4.1 (D5.1 D55) for further details) obtained in the same cortical region and species. Anatomical data of the mouse cortical column at light microscopic level will complete microanatomical data for the model of the mouse somatosensory cortex and will help the functional interpretation of the data obtained from *in vivo* physiological experiments. In addition, data on the ultrastructure of the mouse neocortex are potentially usable for building realistic models of the cortex.

Outputs 3, 4, and 5: These Outputs contribute to the research infrastructure by providing: curated data on proteins present at synapses and protein-protein interactions; detailed information on protein-protein interactions in a standard (rule-based) format; and the means to simulate these interactions in the context of the electrical activity of a neuron.

Output 6: The final version of the integrated environment is in its final development stage and currently can store and operate with multiple input data sources as intended. This Output is being used by neuroscientists in SP1 to improve the workflow of comparative studies and analyses in KR1.5. In addition, Vishnu is designed to allow comparison among multimodal neurological data. With additional changes, it can be extended to work with data from different fields. Furthermore, Vishnu could also be used to pre-process data, for example, by transforming it using the shrinkage factor, to facilitate posterior data comparison.

Output 7: The results related to this Output have been published on BioRxiv (the preprint server for biology, P2346, see Section 6.2.2) and the paper entitled “Calcium channel-dependent induction of long-term synaptic plasticity at excitatory Golgi cell synapses of cerebellum” is under revision. This Output has been used to implement the cerebellar models into the Brain Simulation Platform together with Outputs 4 and 5 of KR1.2.

6.2.2 Publications

Outputs contributing to KR1.4 have contributed to the generation of the publications listed below.

- P2191: Turegano-Lopez, M., Santuy, A., DeFelipe, J., and Merchan-Perez, A. (2019). Size, Shape, and Distribution of Multivesicular Bodies in the Juvenile Rat Somatosensory Cortex: A 3D Electron Microscopy Study. Cereb. Cortex. doi:10.1093/cercor/bhz211.

  Output 1 has been validated by scientific peer review via this publication. In this study, 1618 multivesicular bodies (MVBs) from stacks of FIB/SEM images acquired from the neuropil have been analysed. The density, volume, and spatial distribution of MVBs across the cortical layers of the somatosensory cortex have been analysed. The results show that MVBs are ubiquitous in all components of the neuropil of all cortical layers, indicating that house-keeping functions of

the endosomal pathway are distributed along all kinds of neuronal and glial processes. The data is of interest in computational models of energy metabolism.

- P2192: Slow-wave activity in the S1HL cortex is contributed by different layer-specific field potential sources during development. Tania Ortuño, Victor J. López-Madrona, Julia Makarova, Silvia Tapia-Gonzalez, Alberto Muñoz, Javier DeFelipe, Oscar Herreras, The Journal of Neuroscience, 2019-09-23.

  Output 2 has been validated by scientific peer-review via this publication. Publication results suggest that field potentials are mostly generated by a pathway in deep layers, whereas other pathways mature later in middle layers and take over in adults, suggesting that a functional sensory-motor control relies on a delayed maturation and network integration of synaptic connections in middle layers. Data on the volume occupied by the different elements (neuropil, neurons, glia and blood vessels) will be used to complete microanatomical data for the model of the mouse somatosensory cortex and to help the functional interpretation of the data obtained from *in vivo* physiological experiments of this publication.

- P1551: Architecture of the Mouse Brain Synaptome Fei Zhu, Mélissa Cizeron, Zhen Qiu, Ruth Benavides-Piccione, Maksym V. Kopanitsa, Nathan G. Skene, Babis Koniaris, Javier DeFelipe, Erik Fransén, Noboru H. Komiyama, Seth G.N. Grant (2018) Neuron, Vol. 99, No. 4, Gold OA, Validated http://dx.doi.org/10.1016/j.neuron.2018.07.007

  Output 2 has been validated by scientific peer-review via this publication. In this study, we have integrated molecular and morphological features of billions of synapses to generate synapse catalogs in the whole mouse brain. To perform this study, we have developed genetic labelling and imaging methods to examine synaptic proteins in individual excitatory synapses across all regions of the mouse brain. We have been able to generate single-synapse-resolution maps (synaptome) of postsynaptic proteins across the whole mouse brain. In this article we have found that synaptome mapping of circuits showed correspondence between synapse diversity and structural and functional connectomes. We propose that synaptome technology and resources have wide-ranging application in studies of the normal and diseased brain. These results are crucial for the whole mouse brain modeling.

- P1552: Regional Diversity in the Postsynaptic Proteome of the Mouse Brain Marcia Roy, Oksana Sorokina, Colin McLean, Silvia Tapia-González, Javier DeFelipe, J. Armstrong, Seth Grant (2018) Proteomes, Vol. 6, No. 3, http://dx.doi.org/10.3390/proteomes6030031

  Output 2 has been validated by scientific peer-review via this publication. The aim of this study was to see whether anatomically distinct regions of the mouse brain are characterised by different expression of the entire set of postsynaptic proteins (postsynaptic proteomes). Postsynaptic proteomes were isolated from seven forebrain and hindbrain regions and their composition determined using proteomic mass spectrometry. We found that 74% of proteins showed differential expression and each region displayed a unique compositional signature. Furthermore, combining proteomic and connectomic data we observed that interconnected regions have specific proteome signatures. These results could be used for modelling the whole mouse brain.

- P2146: Dissecting the shared and context-dependent pathways mediated by the p140Cap adaptor protein in cancer and in neurons. J Chapelle, O Sorokina, C McLean, V Salemme, A Alfieri, C Angelini, & al. Frontiers in cell and developmental biology 7, 222.

  This publication confirms that Outputs 4 and 5 are validated by scientific peer review. This publication displays a bioinformatics approach in which the protein interaction network (PPI) is used to implement a molecular integration between neurological and cancer conditions. This article represents one of the first examples of an adaptor protein that participates to biological complexes that are either specific for organs and tissues, or overlapping to both cancer and neurological functions.

- Publications P2346, P2308, P1751, P1657, and P1878 (*in press* during SGA2, published during SGA3) already reported in KR1.2 confirm the validation of Output 7 in this KR. See Section 4.2.2 for further details

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 to meet objective SO1.5: *Obtaining critical information about differences and similarities in brain organisation across species*. In SP1, In addition to the preliminary datasets generated in the first project year, six more main Outputs have been achieved in the second project year. The main Outputs of the second data results package contributing to KR1.5 are outlined below.

7.1.1.1 List of Outputs contributing to this KR

- Output 1: Final datasets of the human neuropil at the ultrastructural level (C1747)
- Output 2: Final 3D reconstructions human neocortical pyramidal cells (C1741)
- Output 3: Final quantitative ultrastructural data of the mouse hippocampus (C1749)
- Output 4: Final datasets to implement comparative studies of mouse and human brains circuits (C2345)
- Output 5: Machine learning-based comparative studies of microanatomy and physiology of mice and humans (C1802)
- Others: Output 6 from KR1.4, Integrated framework for the interactive exploratory analysis of neurological data.

Details of the components contributing to this KR can be found in Annex 1: SP1 Components.

7.1.1.2 How Outputs relate to each other and the Key Result

One of the main aims of SP1 is to obtain critical information about differences and similarities in brain organisation across species. Outputs listed above are related to this aim and they complement each other. Outputs relate to this KR by 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.

All outputs contributing to KR1.5, together with those achieved in the first project year and reported in SGA2 M12 Deliverable D1.6.1 (D7.1 D5), contribute to the SGA2 M24 SP1 Compound Deliverable D1.6.3 (D7.3, D35): Comparative study of cells and microcircuits in the rodent and human brain.

7.1.2 Output 1: Final datasets of the human neuropil at the ultrastructural level

Table 21: Output 1 Links

Component	Link to	URL
C1747	Data Repository	TEC: DOI: https://doi.org/10.25493/QDQJ-V0Z https://kg.ebrains.eu/search/instances/Dataset/cbe4dcac-3fb5-4499-aeec-94f43ed50483 CA1: DOI: https://doi.org/10.25493/NRFB-7N5 https://kg.ebrains.eu/search/instances/Dataset/99faade4-eb61-4007-8ed1-14a5a9392463
	Technical Documentation	'Data descriptor' in the KG
	User Documentation	'Data descriptor' in the KG

The final release of C1747 has been completed. Final 3D Quantitative data of the neuropil at the ultrastructural level from human mesial temporal cortex (neocortex and hippocampus) have been obtained. These new data together with the preliminary dataset released by M12 (see SGA2 M12 Deliverable D1.6.1 (D7.1 D5), Section 7.1, Output 1, for further details) create the full SP1 DMP dataset 'Quantitative data of the neuropil at the ultrastructural level in human brain'. The full dataset comprising 3D properties of synapses has been obtained using FIB/SEM imaging in several regions of the human mesial neocortex from non-pathological human brain tissue. Synapses have been identified, segmented and quantified in the transentorhinal cortex (TEC), and in CA1 hippocampal strata: oriens, pyramidale and lacunosum-moleculare. 7 human cases have been used to achieve a total of 60 FIB/SEM stacks (15 per each region examined). Data obtained include the number and density of asymmetric and symmetric synapses, their sizes and their spatial distribution. The distribution of their post-synaptic targets has also been determined.

Data have been published in the KG by M24 as planned. This Output also contributes to KR1.4; data have contributed to the publication P2139 (Domíngez-Álvaro *et al.*, 2019) (see Section 7.2.2 below)

7.1.3 Output 2: Final 3D reconstructions human neocortical pyramidal cells

Table 22: Output 2 Links

Component	Link to	URL
C1741	Data Repository	DOI: https://doi.org/10.25493/PJG9-ZE6 https://kg.ebrains.eu/search/instances/Dataset/1e87bd86-25fa-43dd-85c6-fc043d4b2687
	Technical Documentation	'Data descriptor' in the KG
	User Documentation	'Data descriptor' in the KG

The final release of C1741 has been completed and data can be considered final. Final quantitative ultrastructural data of the mouse hippocampus have been obtained. These new data, together with the preliminary dataset released by M12 (see SGA2 M12 Deliverable D1.6.1 (D7.1 D5), Section 7.1, Output 1, for further details) form the full SP1 DMP dataset '3D reconstructions human neocortical pyramidal cells'. The full dataset consists of on 155 3D reconstructions of pyramidal cells in human visual cortex (area 17; n= 50), motor cortex (area 4; n= 23), frontal cortex (area 9; n= 30), middle temporal cortex (area 21; n= 17), superior temporal cortex (area 22; n= 7), CA1 distal (transition to subiculum) (n=8) and subiculum (n= 20) of the hippocampal formation, using Neurolucida software on 3D confocal stacks of images.

Data have been published in the KG by M24 as planned. Data from this Output have been published in publication P1963 (see Section 7.2.2). Other publications to which this Output has contributed are P1343 (Eyal *et al*., 2018), 2147 (Tapia-González *et al.,* 2019), 2138 (Mihaljevic *et al*., 2019) and 2380 (Merino-Serrais *et al*., 2020). Datasets not published yet are under embargo until publication.

7.1.4 Output 3: Final quantitative ultrastructural data of the mouse hippocampus

Table 23: Output 3 Links

Component	Link to	URL
C1746	Data Repository	DOI: https://doi.org/10.25493/GHBT-SVN https://kg.ebrains.eu/search/instances/Dataset/5a53361c-aefa-4676-8fab-a272c53b381d
	Technical Documentation	'Data descriptor' in the KG
	User Documentation	'Data descriptor' in the KG

The final release of C1746 has been completed and data can be considered final. Final quantitative ultrastructural data of the mouse hippocampus have been obtained. These new data, together with the preliminary dataset released by M12 (see SGA2 M12 Deliverable D1.6.1 (D7.1 D5), Section 7.1, Output 1, for further details) form the full SP1 DMP dataset 'Quantitative ultrastructural data of the mouse hippocampus'. The full dataset comprises ultrastructural data from 12 FIB/SEM samples of the CA1 area of the mouse hippocampus. The dataset provides densities of synapses in the strata oriens, radiatum and lacunosum-moleculare, distribution on different postsynaptic targets and proportions of excitatory and inhibitory synapses. It has been completed by M24. This Output also contributes to KR1.4.

Data is has been published in the KG by M24 as planned. Datasets are under embargo until publication. This Output also contributes to KR1.4.

7.1.5 Output 4: Full datasets to implement comparative studies of mouse and human brains circuits

Table 24: Output 4 Links

Component	Link to	URL
C2345	Data Repository	https://doi.org/10.25493/93YQ-6QM https://doi.org/10.25493/A4RG-CJN https://doi.org/10.25493/YMV3-45H
	Technical and Documentation	In 'data descriptor' of the above links and at http://dx.doi.org/10.1101/701318 (C2345)
	User Documentation	See above

This Output involves the releases of Component C2345. Data on electrophysiological, morphological and molecular study of layer 6 neuronal microcircuits have been released and used for comparative studies (C2345). See D1.6.3 for detailed information. These data have been generated as the contribution of the Task named *Functional characterization of neuromodulator on the single neuron level in motor and sensory cortices of rodent brain*, T1.5.6 and T2.5.8, in SP1 and SP2 (Human Brain Organization), respectively.

Data from this Output are published in publication P2118 (see Section 7.2.2).

7.1.6 Output 5: Machine learning-based comparative studies of microanatomy and physiology of mice and humans

Table 25: Output 5 Links

Component	Link to	URL
C1802	Model Repository	https://kg.ebrains.eu/search/instances/Model/5ae92dbb-cb01-45be-9eaa-400b54921602 bioArxiv: https://doi.org/10.1101/2020.03.14.991828

This output introduces novel methodology for multivariate comparison and analysis of neuron morphology and electro-physiology data, by analysing compact descriptions of the probability distributions over the morphological and/or electro-physiological features. A paper has been submitted on the morphological comparison between basal dendrites of hippocampus CA1 cells to the open access Scientific Reports journal (P2428). The input to this work was the hippocampus cells data that was generated within KR1.5 by T1.5.1: Machine learning-based comparative studies of microanatomy and physiology of mice and humans (C1740: DOI: https://doi.org/10.25493/H24F-2ET). The model has already been shared with the KG team for its integration. A paper on the electro-physiology and joint morphology and electro-physiology study is planned to be sent out to a journal by the end of M24. Another paper on the comparison of spatial distribution of synapses is currently in preparation. This output has contributed to publications P2428 (Mihaljevic *et al.*, 2020), P1821(Benjumeda *et al.*, 2019), P1722 (Mihaljevic *et al.*, 2018), P1723 (Leguey *et al.*, 2018), P1724 (Antón-Sánchez *et al.*, 2018) and P2138 (Mihaljevic *et al.*, 2019).

7.1.7 Others

Output 6 from KR1.4, *Integrated framework for the interactive exploratory analysis of neurological data*, also contributes to this KR. It is described in detail in Section 6.1.7.

7.2 Validation and Impact

7.2.1 Actual and Potential Use of Output(s)

Outputs 1-3: Datasets have been validated by data users or by data publication. Full datasets on synapses in the neuropil have been completed and are being used in detailed models of the hippocampus and neocortex, as well as in comparative studies between human and rodent brains. In addition, the human data at the light and electron microscopic levels could be used in the Human Brain Atlas. Quantitative data on the ultrastructure of the mouse and human hippocampus are potentially usable for comparative studies. Data from 3D reconstructions of pyramidal cells are being used in models of the hippocampus and neocortex, as well as in comparative studies between human and rodent brains.

Output 4: Datasets generated in this Output have already been published and have been used to implement the comparative studies planned. These studies could be critical to better understanding the similarities and differences of the structural and functional organisation of cortical circuits.

Output 5: This Output introduces a novel methodology for multivariate comparison and analysis of neuron morphology and electro-physiology data, by analysing compact descriptions of the probability distributions over the morphological and/or electro-physiological features. This methodology could be also used outside the HBP by the neuroscience community to perform comparative studies and multilevel approaches to advance in the study of the brain's function.

7.2.2 Publications

Outputs contributing to KR1.5 have contributed to the generation of the publications listed below.

- P2139: 3D Electron Microscopy Study of Synaptic Organization of the Normal Human Transentorhinal Cortex and Its Possible Alterations in Alzheimer's Disease. Domínguez-Álvaro M, Montero-Crespo M, Blazquez-Llorca L, DeFelipe J, Alonso-Nanclares L. eNeuro. 2019 Jul 10;6(4). pii: ENEURO.0140-19.2019. doi: 10.1523/ENEURO.0140-19.2019.

  This publication confirms that Output 1 has been validated by scientific peer review. The present study represents the first attempt to unveil the synaptic organisation of the neuropil of the human brain at the ultrastructural level using 3D electron microscopy (EM). Results provide a new, large, quantitative ultrastructure dataset of the synaptic organisation of the normal human cortex and of the synaptic alterations that occur in Alzheimer's Disease (AD). Thus, these results may help to understand the relationship between alterations of the synaptic circuits and the cognitive deterioration in AD.

- P1963: Differential Structure of Hippocampal CA1 Pyramidal Neurons in the Human and Mouse 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. Cerebral Cortex. http://dx.doi.org/10.1093/cercor/bhz122.

  Output 2 has been validated by scientific peer review via this publication. This publication reveals new differences between rodents and human of some morphological parameters of the pyramidal cells that could be used in comparative modelling of pyramidal neurons in different cortical areas.

- P1343: Human Cortical Pyramidal Neurons: From Spines to Spikes via Models Guy Eyal, Matthijs B. Verhoog, Guilherme Testa-Silva, Yair Deitcher, Ruth Benavides-Piccione, Javier DeFelipe, Christiaan P. J. de Kock, Huibert D. Mansvelder, Idan Segev (2018) Frontiers in Cellular Neuroscience, Vol. 12. http://dx.doi.org/10.3389/fncel.2018.00181

  Output 2 has been validated by scientific peer review via this publication. This study provides the most comprehensive model of any human neuron to-date demonstrating the biophysical and computational distinctiveness of human cortical neurons. Results have been used to implement comparative studies.

- P2147: Differential expression of secretagogin immunostaining in the hippocampal formation and the entorhinal and perirhinal cortices of humans, rats, and mice Silvia Tapia-González Ricardo Insausti Javier DeFelipe (2019) Journal of Comparative Neurology. https://onlinelibrary.wiley.com/doi/full/10.1002/cne.24773

  Output 2 has been validated by scientific peer review via this publication. This study shows remarkable differences in the expression of particular proteins in brain neurons between species. These differences should be taken into consideration when making interpretations in translational studies from mouse to human brains.

- P2380: Calbindin immunostaining in the CA1 hippocampal pyramidal cell layer of the human and mouse: A comparative study. Merino-Serrais P, Tapia-González S, DeFelipe J. 1 Mar 2020. https://doi.org/10.1016/j.jchemneu.2020.101745.

  Output 2 has been validated by scientific peer review via this publication. As in the previous publication, this study shows remarkable differences in the expression of particular proteins in brain neurons in human and mouse that should be taken into consideration when making interpretations in translational studies from mouse to human brains.

- P2118: Cell Type-Specific Modulation of Layer 6A Excitatory Microcircuits by Acetylcholine in Rat Barrel Cortex. Yang D, Günter R, Qi G, Radnikow G, Feldmeyer D. 14 July 2019. DOI: 10.1101/701318.

  Output 4 has been validated by scientific peer review via this publication. This publication provides strategic data to implement comparative studies between rodents and humans regarding the effects of acetylcholine. How the cortex decides to send strong output to one target region, while sending little to another is poorly understood. This study shows that in output layer 6A, this can be regulated by the neuromodulator ACh. The interaction of mAChRs and nAChRs results in an altered excitability and synaptic release, effectively strengthening corticothalamic output while weakening corticocortical synaptic signalling.

- P2138: Classification of GABAergic interneurons by leading neuroscientists. Mihaljevic, B., R. Benavides-Piccione, C. Bielza, P. Larrañaga, and J. DeFelipe. Scientific Data. 2019. https://www.nature.com/articles/s41597-019-0246-8. DOI: 10.6084/m9.figshare.9948803.

  This publication confirms that Output 5 and Output 2 have been validated by scientific peer review. This study presents a classification model in which a dataset containing the classification choices by the neuroscientists according to interneuron type as well as to five prominent morphological features. These data can be used as crisp or soft training labels for learning supervised machine learning interneuron classifiers, while further analyses can try to pinpoint anatomical characteristics that make an interneuron especially difficult or especially easy to classify.

- P2428: Comparing basal dendrite branches in human and mouse hippocampal CA1 pyramidal neurons with Bayesian networks Bojan Mihaljevic,Pedro Larrañaga, Ruth Benavides-Piccione. Javier DeFelipe, Concha Bielza (2020). https://www.biorxiv.org/content/10.1101/2020.03.14.991828v1

  This publication confirms that Output 5 has been validated by scientific peer review. This publication directly addresses the goal of human-mouse comparison of pyramidal neurons and is the implementation of component *Principal cells morphology comparative models* (C1802).

- P1722: Towards a supervised classification of neocortical interneuron morphologies Bojan Mihaljević, Pedro Larrañaga, Ruth Benavides-Piccione, Sean Hill, Javier DeFelipe, Concha Bielza (2018) BMC Bioinformatics, Vol. 19, No. 1. http://dx.doi.org/10.1186/s12859-018-2470-1

- P1723: Patterns of Dendritic Basal Field Orientation of Pyramidal Neurons in the Rat Somatosensory Cortex Ignacio Leguey, Ruth Benavides-Piccione, Concepción Rojo, Pedro Larrañaga, Concha Bielza, Javier DeFelipe (2018) eneuro, Vol. 5, No. 6. http://dx.doi.org/10.1523/eneuro.0142-18.2018

- P1724: A regularity index for dendrites - local statistics of a neuron's input space Laura Anton-Sanchez, Felix Effenberger, Concha Bielza, Pedro Larrañaga, Hermann Cuntz (2018) PLOS Computational Biology, Vol. 14, No. 11, http://dx.doi.org/10.1371/journal.pcbi.1006593

  Publications P1722, P1723 and P1724 confirm that the studies focused on machine learning-based comparative studies of microanatomy and physiology of pyramidal cells in mice and humans (Output 5) have been validated by scientific peer review. These publications address different studies using data generated in SP1 to develop mathematical models and classification methods that could be used to perform comparative studies (P1722), and to carry out anatomical data analysis (P1723) and large scale neural circuit analysis (P1724).

8. Update on implementation of the SP1 DMP

The SP1 Data Management Plan (DMP) has been updated as planned. WP1.6, SP1 Coordination, has been the WP in charge of the Data Management.

The completeness of releases has been assessed according to the timeline planned: at M6 and M12, M18 and M24. As reported in SGA2 M12 Deliverable D1.6.1 (D7.1 D5), the releases planned in the period M1-M12 were achieved as scheduled, except for a minor delay in the first data analysis of the cross-species map of striatal neurons. This issue was mitigated and the preliminary data were released at M22 via SGA2 M18 Deliverable D1.5.1 (D6.1 D56). In the course of the second Project year, the generation of the remaining datasets, models and tools continued as planned. The final update on the implementation of the DMP includes the progress of the components until M24.

SP1 Second Data Results package includes:

- At molecular and subcellular level, new datasets on imaging of Amyloid Beta Oligomers (AbetaOs) as well as full datasets on 2D and 3D mapping of receptors and ion channels in the hippocampus, cerebellum and neocortex have been released to contribute to KR1.1 (Section 3.1) and also to KR1.4 (Section 6.1). In addition, the model "Model of Short and Long-Term Plasticity including NLG/NRXN subcellular pathways" has also been released (see SGA2 M20 Deliverable D1.1.1 (D2.1 D52) for details).
- At cellular and microcircuits level, final morphological and functional datasets have been obtained in the cerebellum, hippocampus, basal ganglia and neocortex. These datasets contribute to KR1.2 (Section4.1), to KR1.4 (Section 6.1).
- At the whole-brain level, new datasets have been obtained on brain structure and function as well as on the vascular system that provides a valuable reference for HBP simulations at brain-wide scale, and that complements data obtained in other parts of the SP1. These releases contribute to KR1.3 (Section 5.1).
- The Outputs achieved for the integration studies include final anatomical and functional datasets in the neocortex (see SGA2 M20 Deliverable D1.4.1 (D5.1 D55) for further details) and cerebellum, as well as new advances for the molecular integration. In addition, a new framework including novel tools for integration, visualisation and analysis of anatomical and functional data will be implemented across different scales. These releases contribute to KR1.4 (Section 6.1).
- Final datasets and models planned to carry out the comparative studies have also been released to contribute to KR1.5. These Outputs include the final datasets on quantitative data of the neuropil at the ultrastructural level, quantitative ultrastructural data of the mouse hippocampus and 3D reconstructions of human neocortical pyramidal cells. In addition, full datasets to implement comparative studies of mouse and human brains circuits. These new data have been used to carry out the comparative studies planned in the current project phase. These studies are reported in detail in SGA2 M24 Deliverable D1.6.3 (D7.3, D35). Finally, novel methodologies for multivariate comparison and analysis of anatomical and functional data have been developed. These releases contribute to KR1.5 (Section 7.1) and also to KR1.4 (Section 6.1).

The datasets generated in the second Project year complement datasets obtained in the first year. At the end of the SGA2, approx. 97% of the planned data have been released and 71% of these data have already been published, 24% are in curation and 4% have not yet been submitted to curation in the KG. The data curation is expected to be finalised by M24-M24+2. A general overview is displayed in the table below.

Table 26: General overview SP1 DMP M1-M24

	Number of datasets released	Published in the KG	In Curation	Not submitted to the Curation Team	WP involved[1]	Percentage of completion at M24 (Datasets generation)
KR1.1	14	6	4	4*	WP1.1	99%
KR1.2	29	17	12	0	WP1.2	99%
KR1.3	13	11	2	0	WP1.3	99%
KR1.4	16 (+8)**	15	1	0	WP1.4, WP1.1 WP1.2 WP1.3	99%
KR1.5	10	6 + 3 in publications	1	0	WP1.5	85%***
TOTAL	**82**	**58**	**20**	**4**	**WP1.1-WP1.5**	**97%**
Models, methods and IT Tools						
	Models	**Methods**	**IT Tools**			
KR1.1	1	3	NA			
KR1.2	1	1	NA			
KR1.3	NA	1	1			
KR1.4	1	1	7			
KR1.5	1	NA	1			
TOTAL	**4**	**6**	**9**			

*** Datasets in this KR have not yet been submitted to the curation team because they are in a patenting process.**

**** A total of 16 datasets have been generated in KR1.4 and 8 additional datasets from WP1.1, WP1.2 and WP1.3 have been used to achieve this KR as planned.**

*****There is a delay in the final release of one of the datasets in WP1.5 due to the Covid19 situation.**

[1]WP involved: see Annex 2: SP1 Objectives and WP structure.

9. Conclusion and Outlook

A total of 28 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 the second project year, it can be considered that SP1 has met the planned objectives (SO1.1-SO1.5, see Annex 2: SP1 Objectives and WP structure) by achieving the five Key Results proposed in line with the SP1 data strategy.

The Outputs included in this Deliverable complement the Outputs outlined in the previous deliverable SGA2 M12 Deliverable D1.6.1 (D7.1 D5). A general overview of the final outputs achieved in SP1 is as follows:

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 has been released, using high resolution analysis of neurons and neuronal microcircuits, both structurally and functionally. New characterisation methods have been 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 by 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 at the NRP (Neurorobotics Platform, together with SP10. This KR is directly linked to CDP1.

For KR1.4, the full datasets obtained are unique datasets of simultaneous recordings of all the major subpopulations of cortical neurons in multiple areas. This is crucial for understanding how the brain works, in terms of single areas and in terms of interactions between multiple areas. The datasets that have been developed are unique for the HBP Multilevel Atlas of the Mouse Brain and are 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.

As in the previous project year, 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-M24. Dissemination activities carried out by SP1 will be displayed in the Periodic Report. 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 has participated and participates in curation, annotation and interpretation of data, which is fundamental to generate a 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 the productivity of such interactions in some cases (e.g. cross-SPs publications such as P1343, P1963, P1962 and P2138).
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 by using Outputs in SP1 described in this Deliverable (KR1.5) 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, they facilitate 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. Further information on these studies can be found in the SP1 Deliverable D1.6.3.

Annex 1: SP1 Components

The components contributing to this Deliverable are displayed in the table below.

KR1.1: High-quality multi-level datasets at molecular and subcellular level of single molecules, single synapses and single cells:

Component ID	Name of the component	Type	Related to WPs*	Final Release
C1767	Nanobodies and other strategies for Next-Generation Brain Imaging & Mapping	Dataset	WP1.1	Brain imaging and mapping: new anatomical patterns of neurological relevant antigens obtained by immunodetection with nanobodies in different areas of mouse and human brains (M24)
C1770	Functional in vivo interaction data between neuroligin and the neuroxin families, and their use for the computational modelling of trans-synaptic signalling	Dataset	WP1.1, WP1.4	Models of Short and Long-Term synaptic plasticity including subcellular pathway; Biochemical and cellular validation of intrabodies (M19); Functional characterization of short- and long-term synaptic plasticity in experimental models of synaptic interference (M19)
C1887	Subcellular 2D and 3D distribution of receptors and ion channels in central cells	Dataset	WP1.1	Subcellular 2D & 3D distribution of K+ (GIRK and SK) channels in the cerebellum and in the hippocampus (M24)

*** Related to WP(s): see Annex 2: SP1 Objectives and WP structure.**

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

Component ID	Name of the component	Type	Related to WPs*	Final Release
C1867	Single-cell 3D reconstruction and measurement of thalamocortical ventral lateral, ventral anterior and parafascicular nuclei	Dataset	WP1.2, WP1.4, WP1.5	Optical microscopy 3D reconstruction and measurement of individually labelled motor thalamocortical cells (final release M24)
C1866	Quantitative ultrastructural analysis of the synapses established by identified thalamocortical axons in specific cortical layers	Dataset	WP1.2, WP1.4, WP1.5	Synaptic and functional diversity of thalamic inputs to sensorimotor cortex and basal ganglia M24
C1774	Structure and function of the striatal matrisomal microcircuit - interneurons and input organisation	Dataset	WP1.2	The striatal microcircuit (M21)

C1775	Structure and function of the striosomal microcircuit in striatum – regulation of dopamine activity	Dataset	WP1.2	The striosomal microcircuit – structure and function (M24)
C1836	The integrated function of the basal ganglia and its control of downstream motor centres – structure and function of pallidal and subthalamic neurons	Dataset	WP1.2	Pallidal and subthalamic neurons within the basala ganglia: Audience: general neuroscience community and internal HBP (M24)
C1771	The micro-connectivity of the cerebellar glomerulus	Dataset	WP1.2, WP1.4	Cerebellar network recorded; Cerebellar neurons recorded (M19)
C1772	Detailed reconstruction of inhibitory interneurons of the cerebellar cortex	Dataset	WP1.2, WP1.4	Electrophysiological data (M13)
C1744	Organisation of dendritic trees of striatal and cerebellar neurons	Dataset	WP1.2, WP1.4	Final data on dendrites of estriatal and cerebellar neurons (M24)
C1743	Organisation of the axon initial segment (AIS) of striatal and cerebellar neurons	Dataset	WP1.2, WP1.4	AIS ion channel expression (M24)

*** Related to WP(s): see Annex 2: SP1 Objectives and WP structure.**

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

Component ID	Name of the component	Type	Related to WPs*	Final Release
C1798	Whole-brain images of neuronal activation related to selected behaviours	Dataset	WP1.1, WP1.4	Images of neuronal activation during different phases of fear memory (M23)
C1797	Whole-brain maps of neuronal activation related to selected behaviours	Dataset	WP1.3	Maps of whole brain with neuronal activation related to selected behaviours (M24)
C1761	Cell localisation methods including cell morphological classification	Software	WP1.3	Cell Localization Methods (M24)
C1765	Cellular resolution calcium activity maps over wide regions of the cortex	Dataset	WP1.3	Wide-field_calcium_imaging_dataset (M24)
C2304	High-resolution optical system for simultaneous imaging and stimulation of neuronal activity over large volumes (mesoscope)	Report	WP1.3	Functional connectivity of cortical neurons on GCaMP6f mice (M24)
C2391	Hybrid platform for behavioural test (grasping, reaching and locomotion)	Report	WP1.3	Hybrid platform for behavioural test (grasping, reaching and locomotion) (M18)

C2303	Sub-cortical recording and manipulation of neuronal activity in awake mice	Dataset	WP1.3	Sub-cortical recording and manipulation of neuronal activity in awake mice (M24)
C2305	Subcortical recording and manipulation of neuronal activity with multipoint-emitting optrodes	Report	WP1.3	Sub-cortical recording and manipulation of neuronal activity in awake mice (M24)

*** Related to WP(s): see Annex 2: SP1 Objectives and WP structure.**

KR1.4: Multi-level datasets generated by integrating neuroanatomical data with genetic, molecular and physiological data using advanced technologies:

Component ID	Name of the component	Type	Related to WPs*	Final Release
C1749	Quantitative ultrastructural data of the mouse neocortex	Dataset	WP1.4, WP1.5	Distribution of synapses on identified neurons (M24)
C1748	Quantitative anatomical data of the mouse cortical column at light microscopic level	Dataset	WP1.4, WP1.5	Final Anatomical Data (M24)
C1611	Integrated Mouse and human synaptic proteome dataset with complete literature/public database coverage	Dataset	WP1.4	~5600 proteins, QC completed (M18)
C1776 & C1777	Molecular rule-based model interactions and parameters pertinent to synaptic plasticity (Merged component ID: C1776 & C1777)	Dataset	WP1.4	Molecular rule-based model interactions and parameters pertinent to synaptic plasticity (M24)
C1612	Rule-based modelling incorporated in Brain Simulation Platform	Software	WP1.4	Simulation demonstrating use of KappaNEURON in Brain simulation platform (M12)
C1869	Interactive tools for the analysis of anatomical and functional data	Software	WP1.4	Interactive analysis toolset (M24)
C1870	Integrated environment for acquisition and early analysis of microanatomical data (components merged id: 1870 & id: 1871)	Software	WP1.4, WP1.5	Integrated Environment (M24)
C1773	The basis of granule cell structural plasticity	Dataset	WP1.4, WP1.2	Granule cells (M24)

*** Related to WP(s): see Annex 2: SP1 Objectives and WP structure.**

KR1.5: Key datasets on single neurons and circuits to be used in comparative studies on human and rodent for modelling. Ground-breaking techniques will be used to generate anatomical and functional datasets. New statistical models will be developed to perform comparative analysis:

Component ID	Name of the component	Type	Related to WPs*	Final Release
C1747	Quantitative data of the neuropil at the ultrastructural level in human brain	Dataset	WP1.5, WP1.4	Synaptic and mitochondrial properties (M24)
C1741	3D reconstructions human neocortical pyramidal cells	Dataset	WP1.5	Dendritic spines of human pyramidal cells (M24)
C1749	Quantitative ultrastructural data of the mouse neocortex	Dataset	WP1.5, WP1.4	Distribution of synapses on identified neurons (M24)
C2345	Quantitative analysis of neuromodular function in the rodent neocortex	Dataset	WP1.5	Preliminary data on neuromodulator subtypes (M24)
C1802	Principal cells morphology comparative models	Model	WP1.5	Final release of the model (M24)

*** Related to WP(s): see Annex 2: SP1 Objectives and WP structure.**

Annex 2: SP1 Objectives and WP structure

SP1 Objectives (taken from SGA2 DoA):

- SO1.1: Generate high-quality molecular/subcellular data needed for hypothesis- and data-driven brain modelling (mostly by SP6, CDP1, and CDP2). The generation of molecular and subcellular datasets, coordinated and integrated with anatomical and functional studies, will provide the foundation for key Platform Deliverables and co-designed driver for advanced Platform capabilities.
- SO1.2: Generate high-quality cellular and microcircuit level data needed for hypothesis and data-driven brain modelling (mostly by SP6, CDP1, and CDP2). A main focus is to integrate anatomical and functional studies on the four main brain regions: neocortex (including thalamus), hippocampus, basal ganglia and cerebellum.
- SO1.3: Obtaining 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 a multiscale and multilevel integration, from microcircuitry up to whole brain level. These data will be fundamental for validation of simulation of brain functionality and connectivity developed within SP4 and SP6. In addition, data from the brain-wide studies will be used to build and validate the simulations of the same experimental paradigms within the NRP, together with SP10.
- SO1.4: Carrying out multiscale investigation on brain physiology and long-range connectivity. Linking detailed anatomical structural data with incomplete light and electron microscopy wiring diagrams and integrate this neuroanatomical information with genetic, molecular and physiological data. This integration would allow generation of models to reason about the data, make predictions and suggest new hypotheses to discover new aspects of the structural and functional organisation of the brain.
- SO1.5: Obtaining critical information about differences and similarities in brain organisation across species. The similarities between the human and mouse in the microanatomy and physiology of pyramidal cells and synapses might be considered as basic building-blocks of cortical organisation and generates a bridge toward SP2 analysis of human brain at the integrative level. In contrast, the differences probably indicate evolutionary adaptations of cells and synapses to particular functions.

Table 27: SP1 Work Package Structure (taken from SGA2 DoA)

Work Package No.	Work Package title	Lead Participant Short name
WP1.1	Subcellular and Molecular	SNS
WP1.2	Cell and Microcircuitry: neocortex, hippocampus, basal ganglia and cerebellum	UNIPV
WP1.3	Whole Brain	LENS
WP1.4	Molecular, anatomical and functional data integration of brain circuits	UPM
WP1.5	Comparative study of cells and microcircuits in the rodent and human brain	VU
WP1.6	Scientific Coordination and Management	UPM