CDP1 Annual Compound Deliverable Year 1 (D1.3.1 - SGA2)

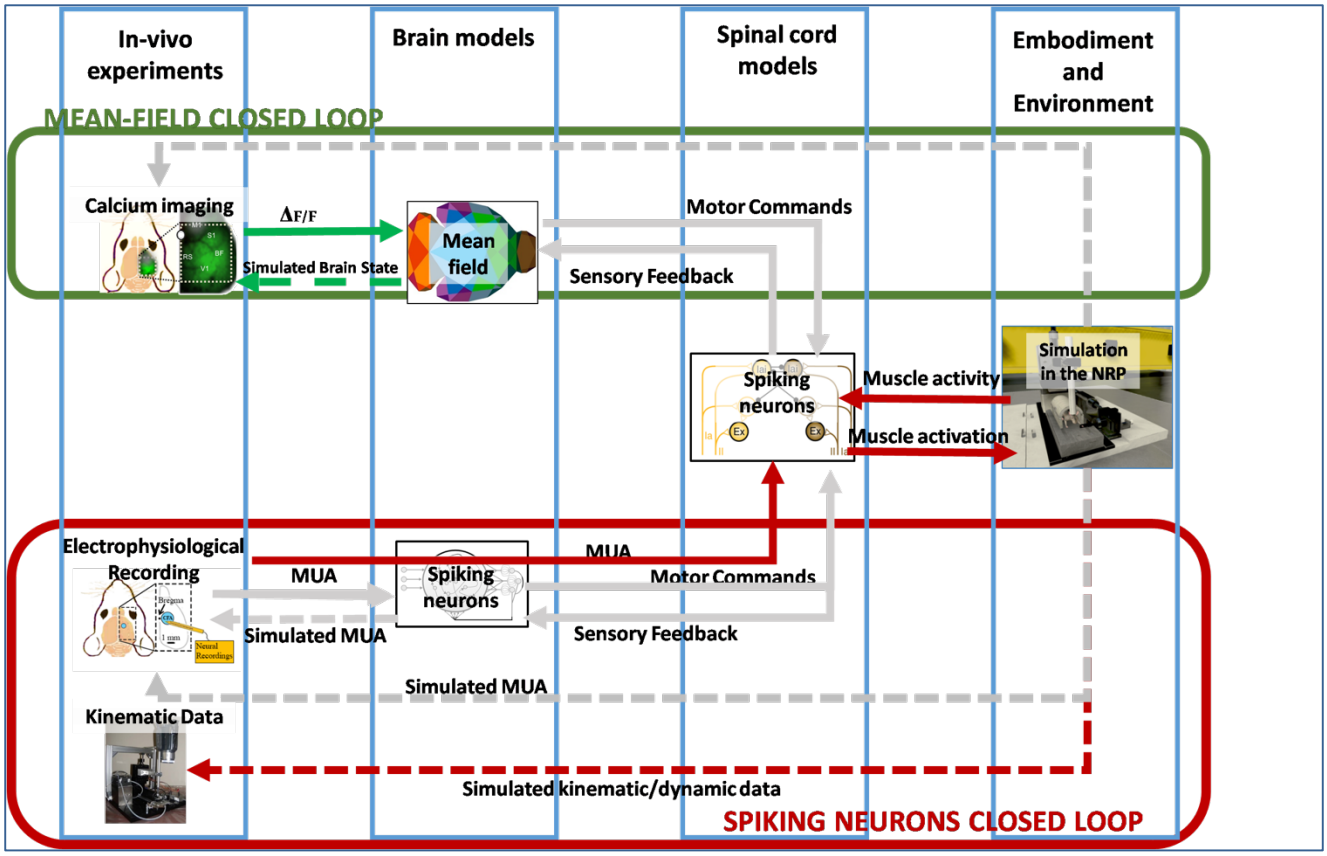

Figure 1: Closed loop experiments in CDP1

Closed loop experiments in CDP1, guiding the development of the Mouse Brain Atlas, HBP Whole Brain simulations and virtual experiments (this figure is from the paper "Experimental and computational study on motor control and recovery after stroke: towards a constructive loop between experimental and virtual embodied neuroscience" by Allegra Mascaro, Falotico, Petkosky *et al*. (under review, submitted in June 2019).

Project Number:	785907	Project Title:	Human Brain Project SGA2

Document Title:	CDP1 Annual Compound Deliverable Year 1
Document Filename:	D1.3.1 (D4.1 D15) SGA2 M12 ACCEPTED 200730.docx
Deliverable Number:	SGA2 D1.3.1 (D4.1, D15)
Deliverable Type:	Report
Work Package(s):	WP1.3, WP4.1, WP4.4, WP4.5, WP5.1, WP5.2, WP5.5, WP6.2, WP7.5, WP10.1, WP10.4
Dissemination Level:	PU = Public
Planned Delivery Date:	SGA2 M12 / 31 Mar 2019, Request for revision: 22 Jul 2019
Actual Delivery Date:	Submitted: SGA2 M13 / 1 Apr 2019, Resubmitted: 24 Oct 2019, Accepted 30 Jul 2020

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Description in GA:	CDP1 Annual Compound Deliverable Year 1 Overview of Key Results and Impact achieved in M1 to M12, tailored for presentation to the relevant audiences (research/industry/public) References to HBP/SP/CDP objectives and Use Cases for navigation between multiple Sub-Projects Linkage of Results to components/a set of component fact sheets (lower level information: Component ownership, Technology Readiness level, performed Quality Control checks, etc.)
Abstract:	The long goal of this Co-Design Project is to integrate contributions from multiple SPs aimed at developing a multi-level model of the whole mouse brain, and the corresponding mouse brain atlas. The workflow proposed by CDP1 is an iterative loop between experiments and simulation, which allows on one side to refine and validate models with experimental data and, on the other side, to redesign experiments based on simulations. This framework will allow neuroscientists to formulate and run their experiments on the HBP Infrastructure and to access and analyse results.
Keywords:	whole mouse brain connectome data, mouse spatio-temporal functional data, mouse multi-level models, rodent virtual behavioural experiments
Target Users/Readers:	Neuroscientific community, Platform users, Clinicians

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History of Changes made to this Deliverable (post Submission)

Date	Change Requested / Change Made / Other Action
1 Apr 2019	Deliverable submitted to EC
22 Jul 2019	Resubmission with specified changes requested in Review Report Main changes requested: 1) extract from Review Report: (1.3, pag. 4 APPENDIX and CORRIGENDUM to Project Review Report) CDP1 Annual compound deliverable of results: a) Clarify how the stroke model (KRc1.2) and the motor task (KRc1.3) are informative for the HBP infrastructure. What is the overall rationale for this research direction within HBP? b) More clear distinctions between the outputs of CDP1 and other related SPs (e.g. SP1) need to be provided. There is a significant overlap in the outputs achieved across CDP and SPs making it very difficult to understand some of the added benefits of this CDP to the overall project c) The outputs of the CDP depend a lot on developments from the BBP. Clarify how HBP has provided results unique relative to the BBP, or what is essential about HBP activities in this CDP relative to those of the HBP. d) The exploitation of the results needs to be clarified and detailed: who, beyond the partners involved in the CDP1, are benefiting from the outputs of this CDP and how? 2) extract from Review Report: (Annex 1, pag 32 of Project Review Report) D4.1 D 1.3.1 CDP1 Annual: Key related components and their location (websites) for accessibility should be included in this report. Adequate and working links to the generated data in a structured format are required.
22 Oct 2019	Revised draft sent by SP/CDP to PCO. Major changes linked to reviewers' requests listed above: 1) See the following sections: a) Overview and new Figure 1 b) Section 3.1.2 and 3.1.3 c) Overview d) Section 3.2.1 and 3.2.2 2) see Annex A: Component Details (Table 1) and Section 3.2.2 Other changes: • Section 4.1.3: eliminated link to a private Collab and insert reference to a publication. • Section 4.2.2: eliminated potential activities related to further developments on Whole brain scaffold model due to activity stop • Section 6.1 and 7: added a sentence related to the amendment of KRc1.4
24 Oct 2019	Revised version resubmitted to EC by PCO via SyGMa

1. Overview

The long goal of this Co-Design Project (CDP) is to integrate contributions from multiple SPs aimed at developing a multi-level model of the whole mouse brain, and the corresponding mouse brain atlas. The workflow proposed by CDP1 is an iterative loop between experiments and simulation, which allows on one side to refine and validate models with experimental data and, on the other side, to redesign experiments based on simulations. This framework will allow neuroscientists to formulate and run their experiments on the HBP Infrastructure and to access and analyse results.

To make these goals tangible, CDP1 focuses on real experiments on mice. These are used as representative paradigms of a larger class of experiments that users can perform using the brain model and the brain atlas on the HBP Platforms. This ensures that the mouse brain model and mouse brain atlas is useful to theoretical and experimental neuroscientists in many contexts.

CDP1 is orchestrating the efforts from various SPs to develop a workflow integrating complementary languages and standards – both on simulation and experimental side. This synergic action is producing a new set of features and capabilities and advance the quality of mouse atlas and mouse brain models together with experimental platforms and data. Experiments chosen to develop such an infrastructure are the motor task and the stroke model, but the final goal is the implementation of the cross-SPs workflow to be extended to a variety of others experiments.

The focal stroke model is a useful paradigm to dissect brain plasticity and connectivity. This is true both from an experimental and modelling side, since it uniquely allows investigating the features of brain activation and brain remapping when a crucial node is detached from the network. This approach is documented in a paper: "*Experimental and computational study on motor control and recovery after stroke: towards a constructive loop between experimental and virtual embodied neuroscience*" by Allegra Mascaro, Falotico, Petkosky *et al*. (under review, submitted in June 2019).

About brain modelling pursued in CDP1: the intent was to use the scaffold whole-brain model developed in SP6 and in the Blue Brain Project that can also be interpreted as a *dynamic brain atlas*, because it not only integrates a large body of data into a consistent system, but also allows to explore its temporal activity dynamics.

Furthermore, by integrating the scaffold whole-brain model into a more and more refined body model of the mouse, we could extend our virtual experimentation capabilities to the domain of awake behaviour experiments. Moreover, it is possible to explore the entire sensory-motor loop in real and virtual experiments.

Being able to perform identical experiments in an animal and *in silico* (simulation) allows us to determine which data or experiment will yield the best improvement of the model and, primarily, will improve our understanding of brain function and dysfunction.

Unfortunately, the team developing the scaffold model (Marc-Oliver GEWALTIG and his group at EPFL, https://collab.humanbrainproject.eu/#/collab/1655/nav/85487) left the HBP in April 2019 and the work of CDP1 on whole-brain modelling has been redirected. Two different strategies are being applied to simulate functional behaviour of the brain in the virtual experiments: a functional model of the motor cortex will be developed by Egidio FALOTICO and his team in SP10, in order to drive the activities of the spinal cord model of the embodied mouse in the virtual environment. In parallel, the whole-brain network model developed by Viktor JIRSA and his team in SP4 will be used for the same purpose. This second option will face the compatibility of mean-field (brain) and spiking neuron (spinal cord) models, a well-known challenge in HBP.

Moreover, the new CDP1 implementation leader, Egidio FALOTICO, seamlessly took over the SP10 coordination role, previously fulfilled by Marc-Oliver GEWALTIG.

2. Introduction

The collaborative framework developed within CDP1 is innovative for two reasons: 1) it merges **cutting-edge activities at the experimental and simulation level**; 2) it allows building a new conception of **closed-loop neuroscience**, where experiments drive simulations and simulations guide experimental design. In this new conceptual framework, experiments are built and validated on theoretical models and virtual platforms, and *vice versa*. In addition, the **tight collaboration between experimental neuroscientists and model developers** is a unique opportunity for a cultural shift, where the experimental paradigm is better constructed based on theoretical predictions. This combined experimental and simulation approach is unique in the wide neuroscientific community and offers the possibility to investigate motor learning on a multitude of levels.

This document shows outputs available for the 4 Key Results (Sections 3, 4, 5 and 6) defined for CDP1 up to now.

In detail, on the experimental side, CDP1 exploits **pioneering imaging techniques** applied to the study of system neuroscience, i.e. light-sheet microscopy, simultaneous large field-of-view imaging and optostimulation in awake animals. Light-sheet microscopy is used to perform **high-resolution mapping** of different neuronal populations in the entire encephalon, providing robust basis to build **realistic models** of rodent brain. We are also **developing tools to understand basic mechanisms of neuronal computation**, e.g. through the investigation on the contribution of different cortical layers or different neuronal compartments to the global signal we record over the entire cortex, or on the modulation of large-scale activity in the transition from deep anaesthesia to awake conditions.

On the software side, existing tools for curating, integrating and exploring rodent data have been consolidated and documented (see publication: Ingvild E. Bjerke, Martin Øvsthus, Krister A. Andersson, Camilla H. Blixhavn, Heidi Kleven, Sharon C. Yates, Maja A. Puchades, Jan G. Bjaalie, Trygve B. Leergaard. *Navigating the murine brain: Towards best practices for determining and documenting neuroanatomical Locations in Experimental Studies*, Front. Neuroanat., 02 November 2018 | https://doi.org/10.3389/fnana.2018.00082: Proposal for best practices for documenting and reporting anatomical locations in experimental studies of mice and rats, demonstrated with use of HBP tools and workflows).

On the simulation side, we created and simulated the **first full-scale** (72 million neurons) **scaffold model of a whole mouse brain**. This brain model is based on whole-brain imaging data from the Allen Brain Institute as well as the Blue Brain Project (SP6). Moreover, the experiments performed in CDP1 are replicated *in silico* on the HBP Platforms, from the single neuron activity in the brain and spinal cord to the mouse musculoskeletal system and to the laboratory environment.

Recently, preliminary high-resolution data on cortical activity recorded *in vivo* have been shared with modellers in SP4 and SP3 to build and validate **models on neuronal activation and propagation** in anaesthetised and awake conditions. These models have been tested and proved to truthfully reproduce brain state transitions and propagation of neuronal activity throughout the cortex.

Resulting validated models will allow to **perform more realistic predictions and simulated experiments on HBP Research Infrastructure**.

In addition, further hardware devices have been developed (e.g. the new R-platform for rehabilitation of rats via robot-assisted reaching). On the *in silico* simulation**, new tools have been developed** (functional model of spinal cord integrated in the virtual mouse within the virtual laboratory environment) **and further implemented into the Neurorobotics Platform**.

3. Key Result KRc1.1 Exploration of spatio-temporal functional and anatomical data

3.1 Outputs

3.1.1 Overview of Outputs

#	Output	Component
1	Storage and curation of CDP1 datasets	C2242, C1765, C1742, C1745
2	Functional connectivity of cortical neurons on GCaMP6f mice	C1765

3.1.2 Output 1: Storage and curation of HBP datasets considered in CDP1

Structural whole-brain datasets are developed in SP1 and made available to build the Whole Brain Atlas and to validate the scaffold model. Functional datasets are conceived both in SP1 and CDP1 (see Section 3.1.3) to validate related brain models and replicated virtual environment in the CDP1 closed loop approach. Datasets are shared among CDP1 teams at first via institutional or HBP Collab private repositories, then stored in HBP archive repositories and processed to be integrated in the Knowledge Graph.

The SP5 curation team has revised the previously used metadata schema to allow more flexible connections between modules. The result of the revised metadata schema – uniMinds – allows all categories of data and models handled to be represented, see SP5 Semester Report M6 (SGA2). The uniMinds is now fully integrated into the Knowledge Graph editor by M12 in SGA2 (See SGA2 Deliverable D5.1.1). Procedures for anchoring of 2D and 3D rodent image data to reference atlases have been optimised. See SGA2 Deliverable D5.2.1.

Several datasets produced in SGA1 SP1 (whole-brain maps of selected neuronal types and maps of neuronal activation) will be publicly available after the embargo, at link1 and link2. Functional imaging of cortical activity after stroke, of which SGA1 datasets have been already curated and integrated in the HBP Mouse Brain Atlas (embargo ended in September 2019) are available at this link. Further details on this dataset are largely documented in a recent publication (Allegra *et al.*, *Cell Reports* 2019).

Further Whole-brain datasets planned in SGA2 from SP1 are:

- *C-Fos datasets (SP1)* – 60 mice in different behavioral conditions. **Timeline**: delivery by December 2019 (aligned to atlas with linear transforms); curation completed by January 2020, embargo until publication (expected submission mid 2020).
- *Cell distribution datasets (SP1)*– 15 mice with different type of interneurons (SST, VIP, PV) labeled. **Timeline**: delivery by Dec 2019 (aligned to atlas with linear transforms); curated by January 2020, embargo until publication (expected submission mid 2020).

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

Functional connectivity of cortical neurons on GCaMP6f mice datasets planned in SGA2 SP1 and CDP1 are:

- *Slow-wave datasets (SP1/CDP1)->* several mice imaged with wide-field and two-photon microscopies for a total of at least 20 datasets. **Timeline**: 16 datasets already acquired; completion of delivery by November 2019; curation completed by December 2019; embargo until publication (expected submission on February 2020).
- *Stroke and rehab datasets (CDP1)->* mice imaged with wide-field and two-photon microscopies during pulling task:
  - *Wide-field datasets:* 3 mice, 1 week before stroke and 4 weeks after stroke (6 datasets). 3 control mice during 4 weeks of training (3 datasets). **Timeline**: completion of delivered by November 2019, curated by December 2019; embargo until publication (expected by the end of SGA2).
  - *Two-photon datasets:* 2 mice, 1 week before stroke and 4 weeks after stroke (4 datasets). **Timeline**: completion of delivered by February 2020, curated by March 2019; embargo until publication (estimated submission mid 2020).

We addressed cortical functional connectivity in anaesthetised and awake mice by performing experiments with wide-field and two-photon microscopy to obtain slow wave datasets. High-resolution (two-photon) microscopy was applied on anaesthetised GCaMP6f mice to validate brain models. These last 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. In detail, we performed data acquisitions under different anaesthesia levels and using different anaesthetics, with both wide-field and two-photon fluorescence imaging. For further details on these datasets, please also refer to SGA2 Deliverable D1.6.1 (D7.1, D5).

3.2 Validation and Impact

3.2.1 Actual Use of Output(s) / Exploitation

The optimised HBP infrastructure and revised metadata curation service allow researchers to share their data in accordance with the F.A.I.R. principles (Findable, Accessible, Interoperable, Reusable). The HBP curation and atlas registration workflows are used by HBP researchers sharing data via the HBP infrastructure, who now have a single entry point for signing their data up for curation: curation-support@humanbrainproject.eu. Non-HBP researchers can exploit the HBP infrastructure through the High Level Support Team, and can request curation service for their data via the HBP web page (https://www.humanbrainproject.eu/en/explore-the-brain/share-data/).

Preliminary datasets on slow waves have been used to validate slow-wave brain model of population activity and also on single neuron, developed in SP4 (DESTEXHE team) and to validate model of slow-wave spatio-temporal pattern of propagation developed in SP3 (PAOLUCCI team).

Preliminary stroke datasets have been used for model implementation and validation by several groups of HBP: DECO team and JIRSA team in SP4, and LASCHI and FALOTICO team in SP10.

Externally to HBP, Prof. FANELLI from the University of Florence is using functional datasets publicly available (DOI: 10.25493/Z9J0-ZZQ) for validation of an inverse model and for analysis of neuronal activity propagation.

3.2.2 Potential Use of Output(s)

Output 1 Storage and curation of CDP1 datasets

All neuroscientists, within and outside of HBP, can take advantage of the HBP Atlas facilities to find and use datasets curated and integrated and/or to share their datasets.

Whole-brain datasets: These datasets were important for the whole-brain scaffold point-neuron model validation. After finalising this modelling work these datasets remain in any case a precious

and unique reference for the entire neuroscientific community. They could be used to explore brain cytoarchitecture, or also to generate and validate whole-brain models at single-neuron resolution, within or outside the HBP.

Output 2 Functional connectivity of cortical neurons on GCaMP6f mice

Validation of brain activity models in the HBP with functional datasets will pursue in SGA3 (WP1). Longitudinal imaging data on stroke and rehabilitated mice will provide a framework to further develop a brain model with predictive capability, ready to be applied in clinical settings to define more effective treatments.

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

3.2.3 Publications

The main publications for this KR are:

1) P829 Ingvild E. Bjerke, Martin Øvsthus, Eszter A. Papp, Sharon C. Yates, Ludovico Silvestri, Julien Fiorilli, Cyriel M.A. Pennartz, Francesco S. Pavone, Maja A. Puchades, Trygve B. Leergaard, Jan G. Bjaalie, *Data integration through brain atlasing: Human Brain Project tools and strategies,* European Psychiatry, Volume 50, 2018, Pages 70-76, ISSN 0924-9338, https://doi.org/10.1016/j.eurpsy.2018.02.004.

   significance: This paper describes the HBP approach to establish an infrastructure for integration of large amounts of heterogeneous neuroscience data

   output supported: #1 Storage and curation of CDP1 datasets

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

   significance: Important example of whole-brain reconstruction of vascular system.

   output supported: #1 Storage and curation of CDP1 datasets

3) P1762 Conti, E., Allegra Mascaro, A. L., Pavone, F. S., *Large Scale Double-Path Illumination System with Split Field of View for the All-Optical Study of Inter-and Intra-Hemispheric Functional Connectivity on Mice,* Methods Protoc. 2019, 2(1), 11; https://doi.org/10.3390/mps2010011

   significance: Technology development to investigate brain plasticity in healthy subjects and after stroke.

   output supported: #1 Storage and curation of CDP1 datasets and #2 Functional connectivity of cortical neurons on GCaMP6f mice

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

1) Bjerke IE, Øvsthus M, Andersson KA, Bjaalie JG, Leergaard TB. Best practices for determining and documenting neuroanatomical locations in the rodent brain. FENS forum of Neuroscience, Berlin 2018

   significance: The HBP workflow for improving assignment of location and documentation to different types of rodent neuroscience data was presented to the European neuroscience community.

   output supported: #1 Storage and curation of CDP1 datasets

2) Blixhavn CH, Andersson KA, Øvsthus M, Bjerke IE, Kleven H, Puchades MA, Leergaard TB, Bjaalie JG. Data integration through digital brain atlasing: Making diverse neuroscience data discoverable and accessible using Human Brain Project infrastructure. FENS forum of Neuroscience, Berlin 2018

   significance: The HBP infrastructure, tools, and workflow for organising, integrating and sharing rodent neuroscience data in line with the FAIR principles was presented to the European neuroscience community.

   output supported: #1 Storage and curation of CDP1 datasets

3) F. RESTA, E. CONTI, E. MONTAGNI, G. DE VITO, A. SCAGLIONE, L. SACCONI, A. ALLEGRA MASCARO, F. PAVONE, *Simultaneous all-optical stimulation and readout of neuronal activity during optogenetically-evoked motor task,* SfN San Diego, 4 November 2018. https://abstractsonline.com/pp8/#!/4649/presentation/41327

   significance: We presented our results in the largest neuroscience audience at the Neuroscience congress, SFN 2018, during the nanosymposium "Voluntary movements".

   output supported: #2 Functional connectivity of cortical neurons in GCaMP6f mice

TOTAL NUMBERS OF DISSEMINATION EVENTS OR OTHER ACTIONS

Output 1: 5

Output 2: 5

4. Key Result KRc1.2 Mouse network model for the activity before and after stroke

4.1 Outputs

4.1.1 Overview of Outputs

#	Output	Component
1	Mouse network model before and after stroke	C1606
2	Model of calcium imaging signals from spikes	C1234, C1235
3	Whole brain scaffold model	C1874, C1877, C3033, C3034

4.1.2 Output 1: Mouse network model before and after stroke

This result consists of a Mouse Brain Model for simulating the rehabilitation experiment defined in CDP1: calcium analysis for 5 mice during 5 weeks (one week before stroke, and 4 weeks of rehabilitation on the M platform).

The brain network model for the resting state activity is built using the open source tracer dataset of the Allen Institute that was implemented into The Virtual Brain (TVB), to obtain detailed Structural Connectivity (SC).

Experimental calcium data are used in a closed loop validation system to model the cortical activity of the mouse. Compared to SGA1 results, the experiment also contains the activity of several regions in the healthy hemisphere, to improve modelling of the dynamical network reorganisation during stroke and recovery.

4.1.3 Output 2: Model of calcium imaging signals from spikes

One of the CDP1 goals is to obtain methods to detect stroke from brain activity. To this end, we use calcium imaging in the mouse to identify how stroke alters brain activity, and use computational models to understand this alteration. One of the first steps is to design models of the calcium imaging signal, and use these models to account for the calcium measurements during normal brain activity. This was done in this first SGA2 year. Preliminary results of these activities are reported in the paper "Experimental and computational study on motor control and recovery after stroke: towards a constructive loop between experimental and virtual embodied neuroscience" by Allegra Mascaro, Falotico, Petkosky *et al.*, (under review, submitted in June 2019).

4.1.4 Output 3: Whole brain scaffold model

(C1874) Our goal during this period was to create a pipeline to systematically generate, simulate and refine data-driven point neuron whole mouse brain models. A first version of the Whole Mouse Brain Cell Atlas was published, together with a web interface to download cell densities, positions and types (https://bbp.epfl.ch/nexus/cell-atlas/, see a screenshot in Figure 2): this public interface presents the Whole Mouse Brain Cell Atlas model, allowing users to navigate through its brain regions to see their cellular composition. Cell counts, positions and densities as well as region meshes can be downloaded and a feedback interface is also available. The latest version of the Whole Mouse Brain point neuron network has been exported in the SONATA format to the BBP Nexus and HBP Knowledge Graph.

(C3033) A database for point neurons parameters was created. It currently hosts the cell types of the whole mouse brain and additional cell-types from Potjans and Diesmann (2014). Literature research was also done to update and refine cell-types distribution of the whole mouse brain model.

(C3034) Another database was created for synaptic parameters. It currently hosts the synaptic types from Markram *et al* (2015) and Potjans and Diesmann (2014). The databases can be extended when additional data become available. They have been integrated into the whole brain generation process.

(C1877) The analysis of the whole brain model has been extended to include simulation of isolated sub-regions and single-cell (Nest) as shown in Figure 3. Results can now be compared to detailed circuit simulations (Neuron).

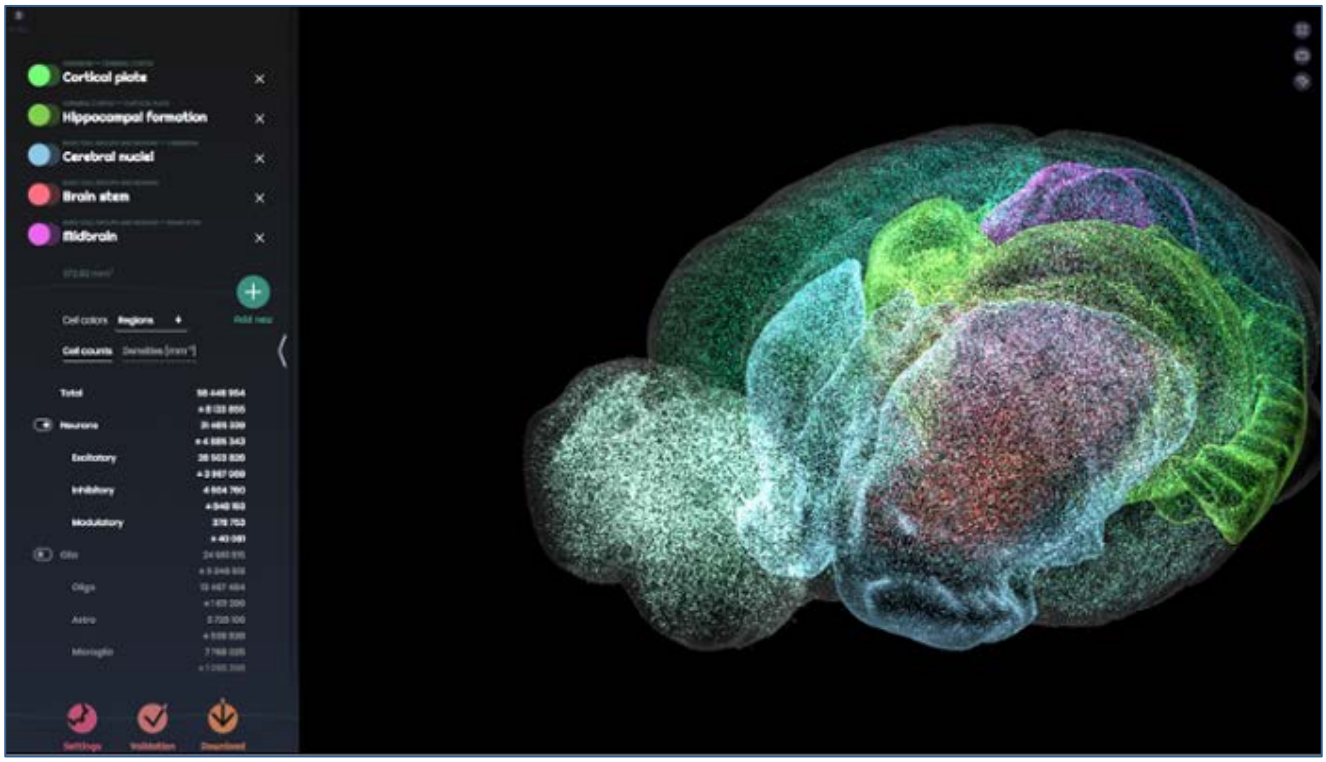


Figure 2: Screenshot of the Blue Brain Cell Atlas

See also: https://bbp.epfl.ch/nexus/cell-atlas/.

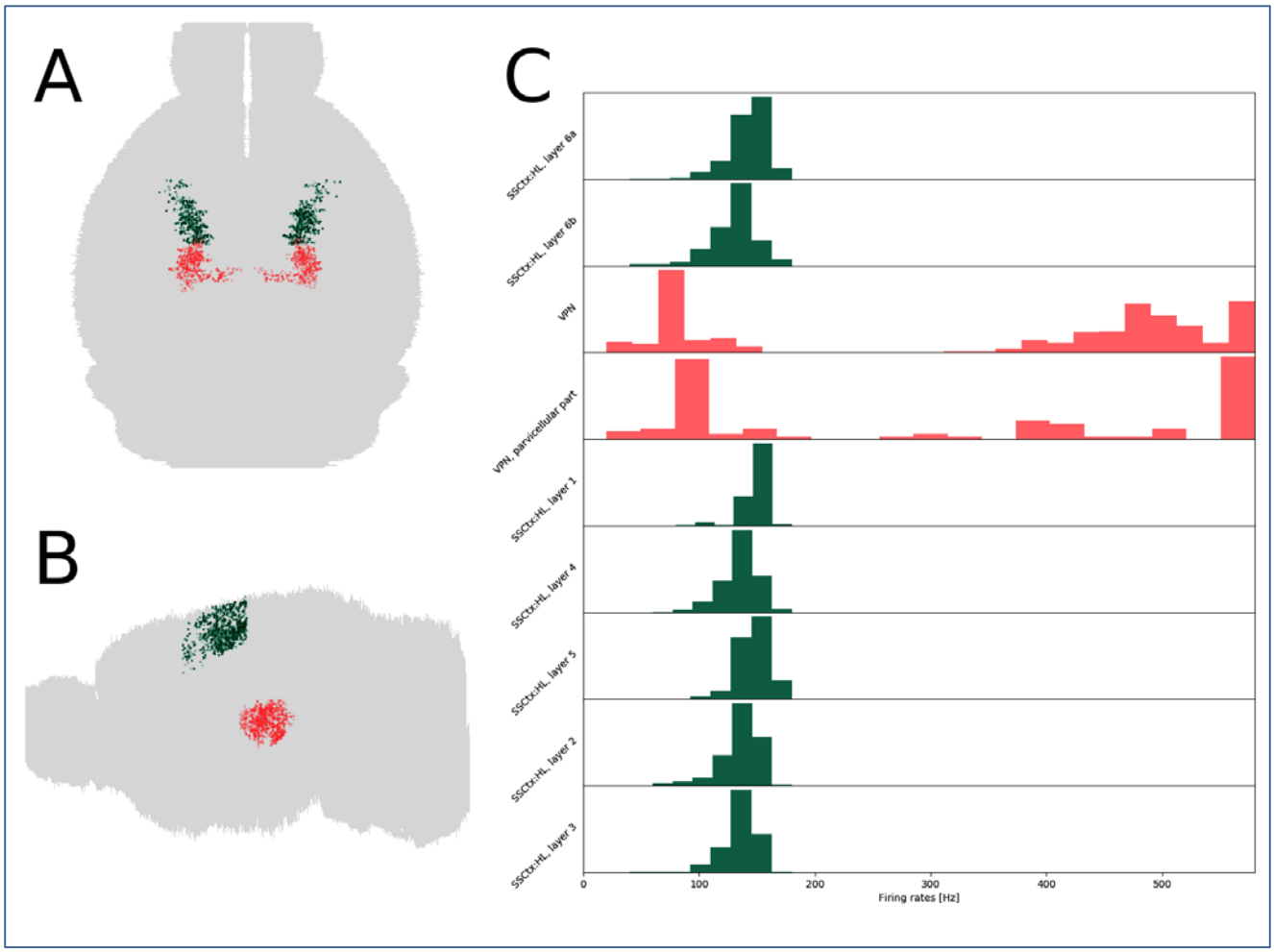


Figure 3: Simulation of subregions of the Whole mouse brain point neuron model

Simulation of subregions of the Whole mouse brain point neuron model (WBM) - A.B.: Axial and sagittal views of the WBM during a simulation of the isolated Primary somatosensory area, lower limb (SSCtx:HL in dark green) and the Ventral posteromedial nucleus of the thalamus (VPN in red). High stimulation of the VPN triggers activity in the SSCtx:HL Neurons and connections of these regions were isolated from the WBM. - C.: Firing rate histogram of the different subregions isolated. This highlights a strong activity in the SSCtx, which will be corrected in a future version of the WBM.

4.2 Validation and Impact

4.2.1 Actual Use of Output(s)

Output 1 Mouse network model before and after stroke

The results of the brain network model of stroke in mice are to be used by the computational and theoretical neuroscience community, as well as experimentalist working on wide-field imaging of rodents. A part of the model is available in the TVB platform which has around 800 new downloads.

The results of the model are of special interest to the wide community using the Allen connectome, since it offers its validation.

Output 2 Model of calcium imaging signals from spikes

The model was able to reproduce the two photon data both at the single cell and at the population level.

Output 3 Whole brain scaffold model

An instance of the Whole Mouse Brain point neuron network Model (WBM) has been integrated in the BBP Nexus and exported to the HBP Knowledge Graph. The Knowledge graph will be the entry point of the SP10 Neurorobotics Platform to access available SP6 models. The WBM was used to benchmark this exchange of models between SP5, SP6 and SP10 and a simplified version was implemented in the Neurorobotics Platform (SP10).

The Mouse Brain Cell Atlas website, despite having been released recently, has already obtained some feedback from the scientific community. This indicates that the Cell Atlas has been used and analysed by other researchers.

A separate version of the Mouse Brain Cell Atlas has been created for the purpose of building the first release of the BBP Mouse Isocortex detailed circuit.

A previous version of the whole-brain model has been delivered to SP9 (UMan).

4.2.2 Potential Use of Output(s)

Output 1 Mouse network model before and after stroke

Developed models allow performing virtual stroke experiments and closer exploring the changes of the function due to the structure, during stroke and recovery. Models will be available on the Collaboratory for HBP and non-HBP members.

Output 2 Model of calcium imaging signals from spikes

This model will be used in the second phase to study the effect of stroke in brain activity.

Output 3 Whole brain scaffold model

The whole mouse brain pipeline project is in structured phase (SP6 model life-cycle). This means that our current objective is to make it reach exploitation phase by validating its different steps. Nonetheless, a first version of the Whole Mouse Brain Cell Atlas has been published and will be used in several projects including the incoming BBP Thalamus project.

Another collaboration has started recently with the SP10 group working on a data-driven reconstruction of the spinal cord to prepare the embodiment of the WBM.

A voucher proposal (#49) was awarded to the Polytechnical University in Milan to develop a simplified cerebellum model, based on the WBM, as well as the detailed cerebellum model, developed in HBP.

4.2.3 Publications

1) P1706 Erö, C., Gewaltig, M. O., Keller, D., & Markram, H. (2018). A Cell Atlas for the Mouse Brain. Frontiers in neuroinformatics, 12, 84.

   significance: For the first time, the mouse cell atlas provides the densities and positions of all excitatory and inhibitory neurons, astrocytes, oligodendrocytes, and microglia in each of the 737 brain regions defined in the Allen Brain Reference Atlas.

   output supported: #3 Whole brain scaffold model

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

Main dissemination activities for this KR:

1) Technology Sheet created for The Virtual Mouse Brain as part of the Technology Map in the HBP technologies. This is then used in the advertisement to the industry and science. The work was also presented by Viktor JIRSA at The 4th Annual BRAIN Initiative Investigators Meeting (Bethesda, USA, 9-11 April 2018).

significance: Bio-medical audience, interested in future health technologies

output supported: #1 Mouse network model before and after stroke

2) PDA Europe, Amsterdam 5 June 2018

significance: Large bio-medical audience, interested in future health technologies.

output supported: #3 Whole brain scaffold model

3) Bernstein's symposium, Univ. Freiburg 19 March 2019

significance: scientific audience at the Bernstein Center Freiburg

output supported: #3 Whole brain scaffold model

TOTAL NUMBERS OF DISSEMINATION EVENTS OR OTHER ACTIONS

Output 1: Mouse network model before and after stroke: 3

Output 2: Model of calcium imaging signals from spikes: 3

Output 3: Whole brain scaffold model: 3

5. Key Result KRc1.3: Implementation and simulation of the motor-task cases in the upgraded virtual behaviour lab app

5.1 Outputs

5.1.1 Overview of Outputs

#	Output	Component
1	Functional model of spinal cord integrated in the Virtual Mouse	C2596, C2599, C2600, C2614, C2615
2	Platform for rat rehabilitation (R-Platform)	C2616
3	First draft of virtual experiment of M-Platform	C2615

5.1.2 Output 1: Functional model of spinal cord integrated in the Virtual Mouse

To fully simulate the stroke rehabilitation procedure, performed with the M-platform on post-stroke mice, several important milestones have to be achieved. A basic component in the simulation is the lower level neural circuit that directly connects with the simulated embodiment, that is the spinal cord circuitry. To produce realistic outputs, such network has to be developed by integrating biologically accurate models of its neural populations. Therefore, we developed a spinal cord circuitry that includes i) a muscle spindle model, capable of reproducing neurophysiological recordings, ii) a model for muscle fibres twitches integration, connected to a population of motoneurons whose membrane parameters are able to implement a specific recruitment order, and iii) interneural populations and excitatory/inhibitory connections that can reproduce monosynaptic and disynaptic stretch/inhibition reflexes.

The spinal network model is illustrated in Figure 4.

Next, we planned to validate the developed spinal cord model, and tune some of its parameters for the generation of appropriate motor commands. Hereto, we devised a validation procedure in which we employ the model on the musculoskeletal mouse embodiment, connected to the M-platform, with the aim of replicating the kinematic movement of the slide. The stimulus to the spinal circuitry is provided by recorded neural activity of relevant neurons of the motor cortex of sane mice whose slide motion is also recorded. Using this data, we can tune the spinal cord model so that the simulated mouse can perform the pulling as closely as possible to its real counterpart (Figure 5).

Currently, reproduction of the movement is not yet accurate, but we identified a possible cause in the variability of firing rates of the recorded neurons. Thus, we are working on a normalisation procedure that can overcome this, by employing multi-unit activity, instead of single-unit. In particular, intracortical voltage signals (sampled at 24 kHz) are computed band-passing recordings in the 300 – 6000 Hz range. The multi-unit activity is calculated from the neural signal, crossing a threshold value defined as three times the standard deviation for each channel. Next, the kinematic signals are oversampled and synchronised with the neural activity (see Figure 6).

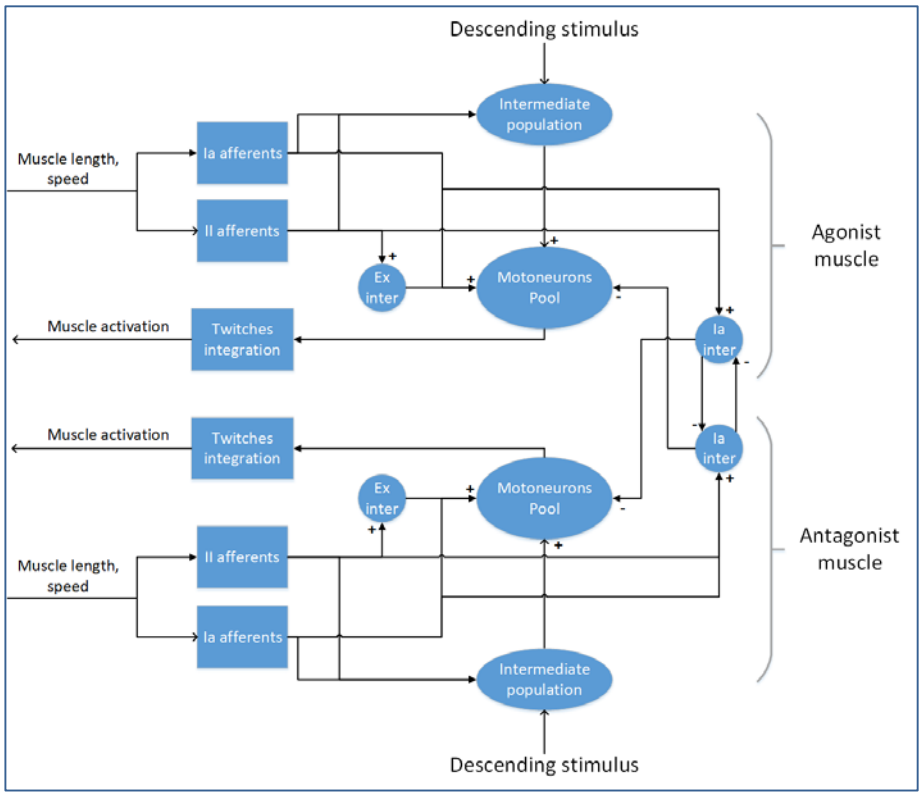
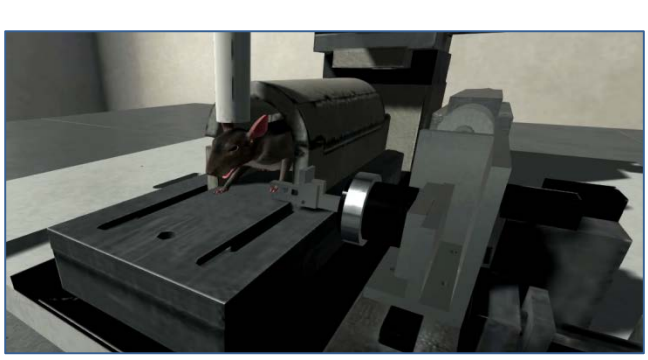


Figure 4: Spinal cord network model.



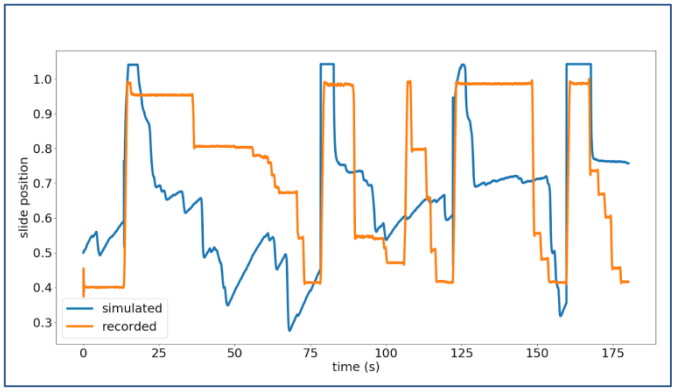


Figure 5: Virtual mouse and M-platform

Simulation of the virtual mouse and the M-platform in the NRP (left). Comparison between slide position recorded during the *in vivo* experiment and the simulated one (right).

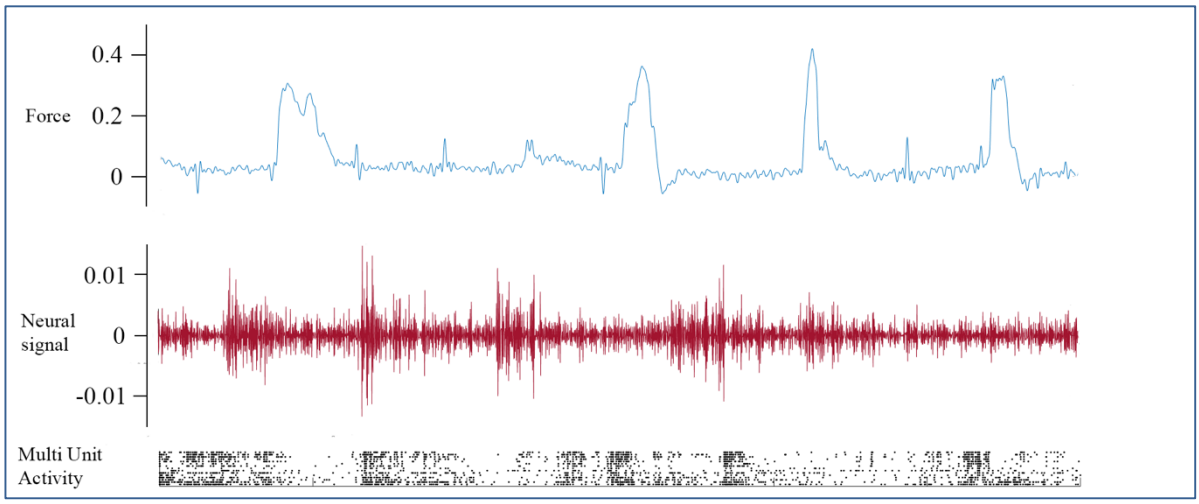


Figure 6: Synchronised neural and kinematic signals.

5.1.3 Output 2: Platform for rat rehabilitation (R-Platform)

In order to perform assisted-reaching experiments, we are developing a new platform for rats (see Figure 7). The device constrains the wrist of the rat and it has 4 degrees of freedom: three space dimensions and the prono-supination movement of the forelimb. In the plane x-y the movement is obtained with a parallel cinematic chain in a 40 x 20 mm2 workspace; an EC-i 40 motor (Maxon) with a spindle drive gearbox allows the movement in the third direction of the space along a track defined by a linear slide (stroke 56 mm). The prono-supination movement is realised with a parallel shift gear mechanism.

This module was integrated with a custom-made restrainer, that allows to fix the rat both in a bipedal and in a four-leg position thanks to a jacket using Velcro. Two translation stages allow regulating the correct posture of the animal compared to the wrist restrainer part, further, two corner braces grant the rotation in three directions of the semi-cylinder component, where the animal is blocked.

This device has two different working modes, a passive modality, where the animal has to perform grasping without help of the robot, and an active mode, where the movement of the paw is completely driven by the robot. The possibility to switch between these modalities allows to follow a rehabilitation process with a customised protocol, moreover, it is possible to introduce a perturbation in the trajectory during a passive grasping of the rat.

During the task, movements are registered with a camera and the force signal is recorded thanks to a 6-axis miniaturised load cell. In the future, EMG signal and neural activity will be added to the recordings in the robot. For immediate future work, experiments will be conducted with three pilot rats to test the performance of the proposed platform.

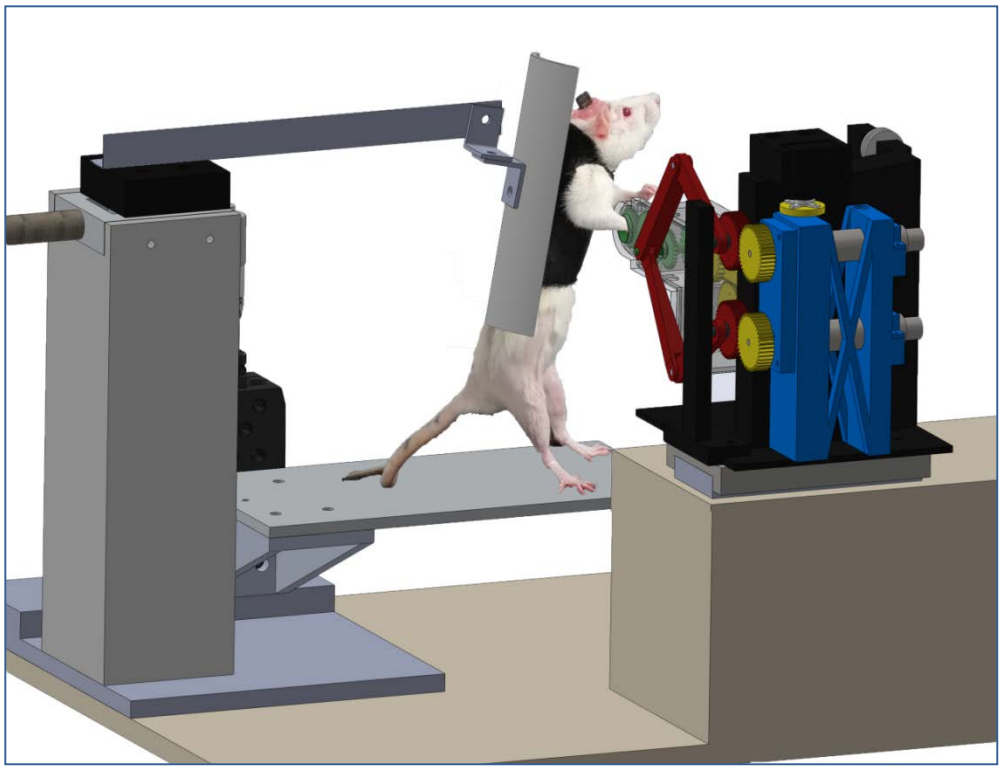


Figure 7: 3D model of the R-platform.

5.1.4 Output 3: First draft of virtual experiment on M-Platform

A preliminary demo of a CDP1 pulling experiment with a spinal cord, muscolo-skeletal system embodied mouse and M-Platform simulations has been deployed on the NRP and is visible on an internal HBP website (http://148.187.97.48/#/esv-private). A snapshot is shown in Figure 8.

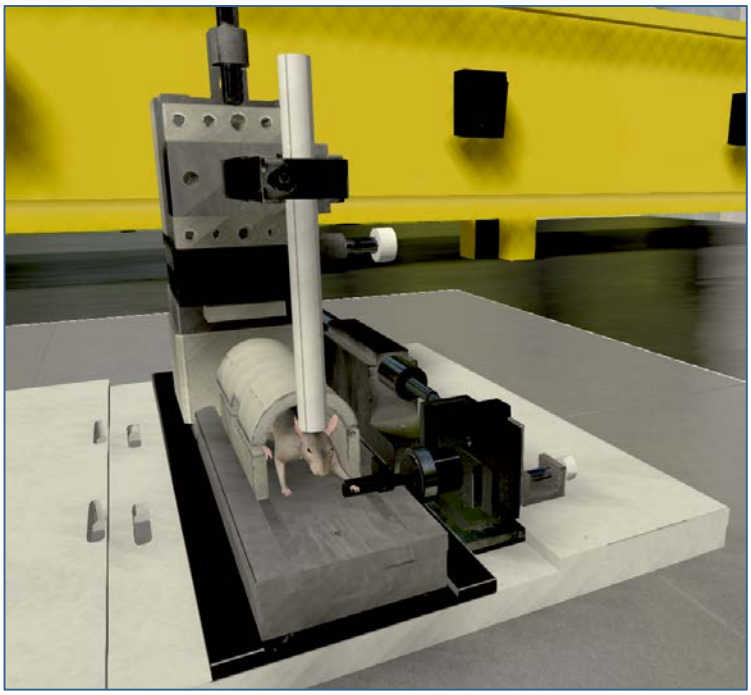


Figure 8: Virtual pulling experiment

5.2 Validation and Impact

5.2.1 Actual Use of Output(s)

Output 1 Functional model of spinal cord integrated in the Virtual Mouse

The outputs of this work are used in the strategic experiments of SP10 and CDP1, to simulate *in vivo* experiments performed by neuroscientists. In particular, one of the experiments aims at replicating the mouse pulling task, before and after a stroke in the motor cortex, including cortical plasticity for rehabilitation, while the other aims at developing patterns for epidural stimulation that can produce grasping motions.

Output 2 Platform for rat rehabilitation (R-Platform)

Results of ongoing experiments are used to settle remaining issues in development of the R-Platform, such as the calibration of the gravity compensation and of the load cell. Moreover, experiments with healthy and injured rats are used to validate the device and to prove the capacity of the platform to detect forelimb weakness.

5.2.2 Potential Use of Output(s)

Output 1 Functional model of spinal cord integrated in the Virtual Mouse

The usage of the outputs is twofold. From one side, it will advance modelling knowledge by testing neuroscientific theories in closed loop with physical simulations. On the other side, experimenters can benefit from the simulations by testing different experimental conditions before performing the real experiment, thus saving time in the experimentation process.

Output 2 Platform for rat rehabilitation (R-Platform)

The new platform will be used to collect kinetic and neural data during a real grasping movement. Recordings can be used to simulate in the NRP the movement of the forelimb. Moreover, it will possible to record the pattern of the neural activity during specific trajectories of the limb.

5.2.3 Publications

1) P1405: Salimi-Nezhad N., Amiri M., Falotico E., Laschi C., A Digital Hardware Realization for Spiking Model of Cutaneous Mechanoreceptor, *Front. Neurosci.,* 2018, https://doi.org/10.3389/fnins.2018.00322

   significance: This model can enrich the mouse virtual body by adding the sense of touch

   output supported: #1

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

1) Egidio Falotico, "Neurorobotics: Robot bodies for artificial brains or brain models for robots?", 3rd HBP Student Conference, 6-7 February 2019, Ghent, Belgium

   significance: Audience mostly PhD students working for HBP, from different backgrounds

   output supported: #1, #3

TOTAL NUMBERS OF DISSEMINATION EVENTS OR OTHER ACTIONS
Output 1: 1
Output 2: -
Output 3: 1

6. Key Result KRc1.4 Validation workflow for virtual experiments on mouse stroke toward a comparison across species

6.1 Outputs

A physical kick-off meeting has been held at LENS (10-11 September 2018), involving leaders and/or researchers of several Work Packages, from Subprojects involved in CDP1 (SP1, SP2, SP3, SP4, SP6 and SP10) and some other CDPs (CDP2, CDP4, and CDP8) and Maurizio CORBETTA from the HBP Clinical Advisory Board. During the meeting, different observation and rehabilitation techniques applied in mouse, human and NHP (Non-Human Primate) have been explored, and a draft plan for a possible continuation of CDP1 in SGA3 has been produced: a roadmap on experimental and common simulation strategies, applicable to both Human and Mouse in stroke conditions, toward a framework for comparison of rehabilitation across species.

At the date of writing of this document, March 2019, there is still no usable output for KRc1.4.

This KRc will be cancelled in the next Amendment of SGA2 due to non-feasibility.

7. Conclusion and Outlook

CDP1 has continued in the closed-loop approach in helping the HBP Platform development, making ad hoc real experiments to obtain datasets for validating models and simulations and to realise the virtual replications of a set of experiments with mice. We demonstrated the technological progress we developed in CDP1, with the purposes of investigating brain plasticity and improving the mouse brain atlas. Recently, CDP1 enriched the spatio-temporal functional and anatomical datasets and models. In detail, (i) the mouse whole brain atlas has been further improved with new cell-distribution datasets, (ii) functional cortical imaging has been extended to both hemispheres, and integrated with high-resolution two-photon data.

On the experimental side, we used optical imaging techniques combined with genetically encoded markers and functional indicators to address how functional recovery of goal-directed motor skills after stroke is reflected in cortical remapping. Currently, we are acquiring experimental datasets on mice performing a reach-to-grasp motor task, as planned, and realising the corresponding virtual experiment. As planned, we are analysing cortical activity with multiple modalities, either with high resolution, small field of view, or low resolution but on the meso-scale, that will be useful for merging multi-level simulations of the mouse brain.

Compared to SGA1 results, now, our experiments also include the activity of several regions in the healthy hemisphere, thus allowing better modelling of the dynamical network reorganisation during stroke and recovery. In addition, high resolution two-photon calcium imaging is now being used to complement large-scale functional measures, and this will be an essential tool to bridge large-scale (mean field) models with simulation of smaller networks with cellular resolution. Finally, first models of slow-wave activity, built and validated on our functional data, are providing the basis to understand the abnormal functionality in perilesioned brain areas.

We have been delayed on the experiment on subcortical recording and simultaneous optogenetic stimulation of awake mice due to bureaucratic issues, but we are now getting on track with the first characterisations of the optrodes.

On the anatomical mapping, an important example of whole-brain reconstruction of the vascular system has been provided and the associated data will be publicly available in months M16-M20. The whole brain scaffold model has been further refined to obtain better results in the simulation experiments.

Workflows and protocols used for documenting and reporting anatomical locations in experimental studies of mice and rats have been published: all users, HBP and externals, can adopt them for sharing and exploring datasets.

A novel longitudinal analysis from new datasets on rehabilitated mice has started for validation of the predictive capability of the Mouse network model. Development of the model for embedding of spiking model modules into the whole brain model has started. Development of a large-scale brain network model for integration in the Virtual Mouse Model used in the NRP *in silico* experiments, as alternative for the whole brain scaffold model, has started.

Refinement of the whole mouse brain scaffold model is ongoing; a standardisation and an optimisation of the Whole Mouse Brain Modelling Workflow was performed to systematically generate and analyse whole brain models.

The experiments are simulated in their entirety on the HBP Platforms (Neuroinformatics - NIP, Brain Simulation - BSP and Neurorobotics - NRP) to gain further mechanistic insights into cortical plasticity and recovery from the virtual experiment. In detail, experimental recordings of neuronal activity (both calcium wide-field and Local Field Potential), simultaneously with the forces applied during a goal-directed motor task, are used in the closed-loop validation system to model the entire physical experiment within a virtual environment. The implementation of the virtual experiment with an embodied mouse and rat in a simulated environment will help in reducing the amount of experimental animals.

In the second year of SGA2, we will provide more datasets on high-resolution imaging of neuronal activity in anaesthetised and awake mice. We will further refine models of calcium and slow-wave activity and test their efficacy in simulating the peri-lesional activity of stroke mice. We will also provide subcortical recordings in parallel with optogenetic stimulation via new-generation optrodes.

The virtual environment realised for mouse behavioural experiments (M-Platform) will be replicated for rat rehabilitation (R-Platform). A pilot simulation of the grasping experiment shall be delivered by the next year.

The activity related to a comparison of stroke and rehabilitation strategies across species (KRc1.4) has been started. This will enable reliable translational studies on stroke that will advance our knowledge and drive the development of new treatments for stroke in humans.

Annex A: Component Details

Table 1: Component details related to Key Result KRc1.1

ID	Component Name	Type	Contact	Additional information
C2242	SGA2_T5.2.2_2242: Optimized procedures for anchoring of 2D and 3D image data to reference atlas	report	Trygve LEERGAARD	Release: Report D5.2.1 submitted Effective Date: M12 https://www.humanbrainproject.eu/en/explore-the-brain/search/
C1765	Cellular resolution calcium activity maps over wide regions of the cortex C1.3.4.1	dataset	Anna Letizia ALLEGRA MASCARO	*Slow-wave datasets*: two photon and wide-field imaging data of GCaMP6f mice in resting state. *Stroke and rehab datasets*: two photon and wide-field imaging data of GCaMP6f mice during pulling task.
C1742	Whole-brain images of different molecular/transgenic markers	dataset	Ludovico SILVESTRI	DOI: 10.25493/77F8-7B4
C1745	Whole-brain maps of different neuronal types	dataset	Ludovico SILVESTRI	DOI: 10.25493/68S1-9R1

Table 2: Component details related to Key Result KRc1.2

ID	Component Name	Type	Contact	Additional information
C1606	SP4 - SGA2 - Mouse stroke brain network model	model	Viktor JIRSA Spase PETKOSKI	Description: model of the effect of the stroke on the large-scale functional brain connectivity using the structural data from the Allen Institute and the empirical data from calcium imaging Model Catalog: link to model implemented in TVBcollab that runs in The Virtual Brain
C2495	SP4 SGA2 Compare experimental and theoretical data: mouse resting state functional connectivity	model	Viktor JIRSA Spase PETKOSKI	Description: Empirical mouse data (calcium, Task T4.1.4) for the resting state functional connectivity, modelled and validated using the structural connectome of the Allen Institute
C998	Allen Mouse Brain Atlas-based brain network	model	Viktor JIRSA Spase PETKOSKI	Description: model of connectome of the mouse brain on diffusion MRI data, based on tracer datasets from the Allen Institute for Brain Science
C1235	Local network model of spontaneous activity in cortex	model	Alain DESTEXHE Núria TORT-COLET	Description: Biophysically plausible models of spontaneous activity and slow-waves by AdEx networks
C1234	Model of calcium imaging signals	model	Alain DESTEXHE Núria TORT-COLET	Description: Biophysically based model of calcium imaging signals from empirical data
C1874	SGA2 - T6.2.6 - whole brain scaffold	other	Marc-Oliver GEWALTIG Dimitri RODARIE	Description: Meta-Component to group all Components related to the whole brain scaffold. Downstream Components will generally refer to this meta-Component to address the whole

				brain scaffold, rather than listing all parts of it Release: version 10 Effective date: 01/03/2019 Model Catalog: https://collab.humanbrainproject.eu/#/collab/1655/nav/75901?state=model.07a61338-2b63-45f8-b790-cf0ab533070f
C1877	SGA2 - T6.2.6 - point-neuron level simulation	service	Marc-Oliver GEWALTIG Dimitri RODARIE	Description: Data formats and APIs to access and simulate the whole brain scaffold in the HBP Collaboratory
C3033	Point neuron electrical parameters database	dataset	Marc-Oliver GEWALTIG Dimitri RODARIE	Description: This database gathers the electrical properties of the point neuron models used to generate and simulate point-neuron networks inside the HBP (whole mouse brain, etc.), as well as simplified cell distributions across brain region
C3034	Synaptic parameters database	dataset	Marc-Oliver GEWALTIG Dimitri RODARIE	Description: This database is gathering the electrical properties of the synaptic models used to generate and simulate point-neuron networks inside the HBP (whole mouse brain, etc.), as well as simplified connectivity recipes linked to the point neuron database

Table 3: Component details related to Key Result KRc1.3

ID	Component Name	Type	Contact	Info on releases and major updates
C2596	SGA2-C10.1.1.1 Virtual mouse	service	Marc-Oliver GEWALTIG Egidio FALOTICO	Description: Meta-component that combines all parts of the virtual mouse
C2599	SGA2-C10.1.1.4 Cortico-spinal integration base model	model	Marc-Oliver GEWALTIG Egidio FALOTICO	Description: generic model for the integration of descending supra-spinal commands with spinal cord sensorimotor circuits models for the virtual mouse
C2600	SGA2-C10.1.1.5 Neuromuscular integration of spinal cord base model	model	Auke IJSPEERT Egidio FALOTICO	Description: integrates the spinal cord-model developed in SGA1 and enhanced in SGA2 with the virtual mouse musculoskeletal model and sensory models
C2614	SGA2-C10.1.4.1 Virtual robotic experiment for stroke rehabilitation	software	Cecilia LASCHI Egidio FALOTICO	Description: the software simulates cortical damage and possible re-organisation of cortical connection/activities
C2615	SGA2-C10.1.4.2 Robot-based training in rodents: pulling experiment	report	Silvestro MICERA Egidio FALOTICO	Description: the report will report about experiments performed by the robot in respect to the real ones
C2616	SGA2-C10.1.4.3 Robot-based training in rodents: assisted-reaching experiment	hardware	Silvestro MICERA Maria PASQUINI	Description: realisation of the Rodent Platform to perform physical assisted-reaching experiments