Human Brain Project

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Abstract:	This report describes HBP Subproject 2 “Strategic Human Brain Data” SP2, its relations with other Subprojects and the work that it will perform in the HBP ramp-up phase. It also sets out the metrics that will be used to measure progress.

Keywords:	Strategic human brain data, Human Brain Atlas, multi-level organisation, Key Performance Indicators

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1. Executive Summary

The Strategic Human Brain Data Subproject (SP2) plays a vital role in the HBP. Its goal is to provide data from *post mortem* and *in vivo* imaging studies on the multi-level mapping of the human brain, as a prerequisite to understanding the rules of human brain organisation. This document provides an overview of SP2 and a detailed description of the work it will perform during the HBP ramp-up phase.

SP2's data will provide essential inputs for the Human Brain Atlas and Brainpedia that are being implemented in the Neuroinformatics Platform (SP5) and make possible the brain modelling tools that the Brain Simulation Platform (SP6) is delivering. SP2's data are also vital for the Medical Informatics Platform being constructed by SP8, as the diseased brain can only be understood by seeing how it differs from the "normal" brain organisation. SP2 depends on SP7's High Performance Computing Platform and works closely with external partners like the Allen Brain Institute.

SP2's tasks in the ramp-up-phase are spread across two areas. In the first area of work, SP2 will investigate the different levels of organisation of the human brain. The second will consist of activities related to data aggregation, analysis and dissemination. Within these tasks, the work will be organised in 4 stages: method development, strategic data acquisition, integration of data, and deposition of data in the HBP Atlas. Progress will be monitored using milestones, indicators and target values described later in this document.

In April 2014, new partners will join SP2 as a result of the HBP competitive call. Additional administrative support has been put in place to ensure they are fully integrated in the work process.

2. Introduction

2.1 The Human Brain Project (HBP)

The goal of the Human Brain Project (HBP) is: "to build a completely new ICT infrastructure for neuroscience, and for brain-related research in medicine and computing, catalysing a global collaborative effort to understand the human brain and its diseases and ultimately to emulate its computational capabilities."

The HBP is based on three principal pillars: Data, Theory and ICT Platforms. Data subprojects will generate “strategic data” to fill critical gaps in our experimental knowledge of the brain’s structure and functioning. These data will be used to refine our theoretical understanding of the brain and to populate specialised ICT Platforms for neuroinformatics (including brain atlases and a “brainpedia”), brain simulation, medical informatics (centralising information on brain diseases), neuromorphic computing (ITC which mimics the functioning of the brain) and neurorobotics (allowing virtual testing of brain models and simulations). These platforms will be supported by a High Performance Computing Platform.

2.2 About this document

This document is the first formal Deliverable from the HBP’s Strategic Human Brain Data Subproject (SP2). After providing an overview of the Subproject and its overall goals, this document will describe in detail SP2’s work process in the HBP’s ramp-up phase (2012-16) and the Key Performance Indicators that will be used to measure progress.

SP2’s Tasks in the ramp-up-phase are broadly divided into two main categories: those concerned with different levels of organisation in the human brain, and those that focus on data aggregation, analysis and dissemination. Within each task, the work will be organised in four distinct stages: method development, strategic data acquisition, integration of data and deposition of data in the HBP Atlas.

2.3 The HBP’s Strategic Human Brain Data Subproject

SP2 generates strategic multi-level data about the human brain that parallel the data collected for mouse brain in the HBP’s Strategic Mouse Brain Data Subproject (SP1). This effort involves understanding how the human brain is organised on different levels, analysing the relationships between different types of data characterising brain organisation, and selecting those that are strategically relevant for building models. We expect to observe significant differences with respect to the mouse data, in particular concerning cognitive circuits (e.g. the circuits responsible for language, symbolic representation and number processing), the distribution of white matter, and patterns of cortical segregation. Other levels of organisation—e.g., cellular, sub-cellular and molecular—are expected to be more similar.

For technical, practical and ethical reasons, it is harder to collect data from the human brain than from rodents. Some methods (e.g. retrograde tracing, some electron microscopic and optogenetic techniques) cannot be applied to the human brain for technical reasons. Other factors causing practical difficulties include the sheer size of the

human brain, the complex folding of the human cerebral cortex, and the impossibility of assembling the large samples that would be needed to account for inter-subject variability, which is higher than in mice. Finally, ethical concerns have led to the definition of regulatory standards for the acquisition and processing of brains from human donors, and for imaging experiments on the living human brain, that are generally far more restrictive than those governing experiments on animal brains. As a consequence, our knowledge of the neurobiological basis of human brain organisation is incomplete. Mouse brain data can be used to generate hypotheses about the organisation of the human brain, but adequate human data remains critically important for human brain modelling in the operational phase of the HBP.

To fill the gaps, SP2 will generate and aggregate strategic data from different levels of human brain organisation:

1) Anatomical and functional organisation
2) Connections between brain regions on multiple levels
3) Numbers and distributions of neurons and glia in defined regions,
4) Morphologies of neurons in different regions,
5) Distribution of selected receptors in the human brain,
6) The developing infant brain.

SP2 will combine data from *post mortem* studies with data from non-invasive imaging studies of the living human brain. Integrating Strategic Mouse Data from SP1 and insights into Cognitive Architectures from SP3 will make it possible to derive general principles of organisation. Other HBP Subprojects will use these principles to predict features of the human brain that have not yet been measured experimentally, or which are not experimentally accessible at all.

The human data to be generated by SP2 will complement existing data. They represent a small, but highly important, fraction of the data required to model the human brain. For example, our knowledge about the connectivity structure of the human brain at the microscopic level is highly limited, which makes it difficult or sometimes even impossible to interpret diffusion-based data from *in vivo* analyses at the microscopic scale. Data on the distribution of neurons in the cerebral cortex in the different cortical areas are very limited. However, such data is needed to indicate the extent to which data from the mouse brain can be applied for human brain simulation. A last example concerns the morphology of human neurons, which has been shown to be different from that of mouse neurons, with implications for the physiological properties of neurons and small circuits, and for models (see 3.1.4).

We will derive general principles describing the structural organisation of the human brain – allowing predictive reconstruction of human brain models. To reach this goal in the operational phase, the aim in the ramp up-phase is to develop the workflows required to generate, analyse and share the data, and to ensure that the methods used and data generated meet the highest possible quality standards and HBP requirements.

2.4 SP2's interactions with other HBP SPs

SP2's data will make critical contributions to the HBP in a number of ways. They will provide essential inputs for the Human Brain Atlas and Brainpedia that are being implemented in the Neuroinformatics Platform (SP5). They will also make a fundamental contribution to the human brain models and simulations that SP6 will develop in the operational phase. SP2's data are also vital for the Medical Informatics Platform being constructed by SP8, as the diseased brain can only be understood by seeing how its organisation differs from that of the healthy brain. This understanding should ultimately lead to new, more effective diagnostic tools and therapies. The High Performance Computing Platform is a key partner for analysing, handling and visualising the data obtained in SP2, and "big data" analysis will play a crucial role in human brain investigations. For an overview of the structure of SP2 and its partner SPs, see Figure 1.

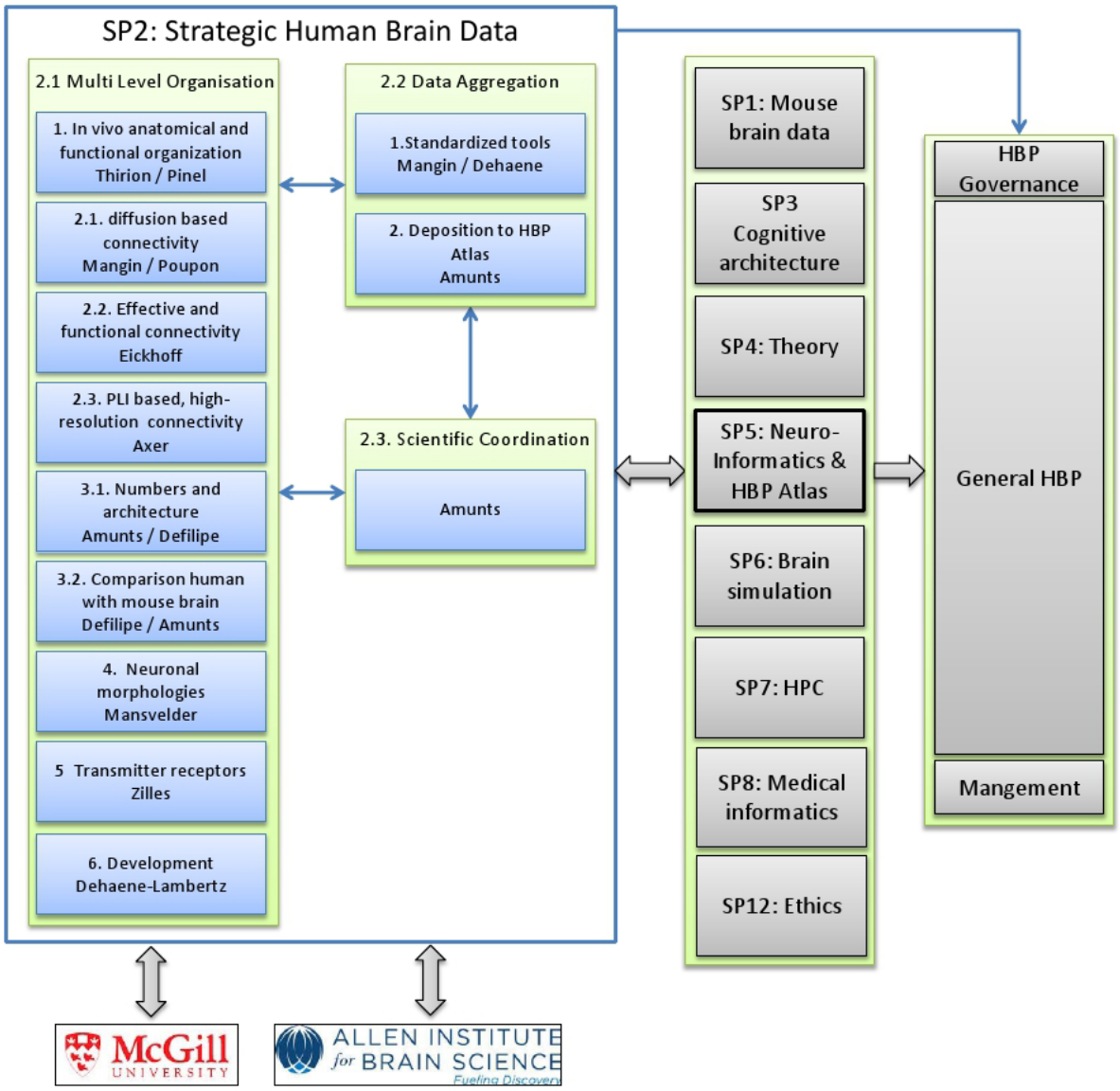

Figure 1: Interaction between SP2 and its direct partner Subprojects.

3. Organisation

SP2 is led by Katrin Amunts at JUELICH/UDUS, Germany, and Jean-Francois Mangin at CEA, France. SP2 is divided into three Work Packages (WPs) and nine Tasks. It has to reach 24 Milestones and generate four Deliverables. Two tasks combine several different activities, and can be further subdivided (Figure 1). The partners come from five institutions based in four countries. SP2 is closely connected to SP1, SP3, SP4, SP5, SP6, SP7, SP8 and SP12 (see Figure 1).

3.1 WP 2.1 Multi-level organisation of the human brain

WP 2.1 and its six component tasks generate strategic, multi-level information about the structure of the human brain, using high-resolution analysis of *post mortem* brains, and innovative neuroimaging techniques of living human brains. Thus, they link microstructural organisation principles with those of cognitive architecture. They also provide the basis for comparative studies.

3.2 WP 2.2 Data aggregation, analysis and dissemination

WP 2.2 and its two component tasks will ensure that the data generated in WP 2.1 are integrated into the HBP Brain Atlas, being compiled by SP5 in its Neuroinformatics Platform. They will also develop the tools needed to achieve this goal, facilitating integration with mouse data from SP1 (Strategic Mouse Brain Data), *in vivo* imaging data from SP3 (Cognitive Architectures) and SP8 (Medical Informatics Platform).

3.3 WP 2.3 Strategic Human Brain Data: scientific coordination

WP 2.3 will sure that the methods used and the data generated meet HBP requirements and quality standards, and summarise results in reports.

3.4 Milestones & Deliverables

SP2 has 24 Milestones and four Deliverables. All tasks began in Month 0 and will continue until Month 30. The allocated Budget for this period amounts to EUR 1.5 million. UDUS has hired a coordinator for the administration of the SP and has set up a schedule of regular virtual and physical meetings within SP2, with the other SPs and with appropriate external partners.

3.5 Geographical distribution and SP expansion

SP2 includes partners from five European countries: Belgium, France, Germany, Netherlands and Spain. In April 2014, five new partners from France (2), Netherlands (2) and Belgium (1), will join following their successful application to the HBP Competitive Call. They will add vital contributions to WP 2.1 and WP 2.2 and increase SP2's overall budget by nearly 60%. The new partners' roles will be described in detail in the second SP2 Deliverable, which is due in Month 12.

3.6 Key external partners

SP2 works with two important external partners: the Allen Institute for Brain Research in Seattle, USA, which provides atlas data on gene expression in the human brain, and the Brain Imaging Centre of the McGill University, Montreal, Canada, which contributes methods and routines to analyse the BigBrain data set.

4. Work process: different levels of brain organisation

This chapter describes work that will be performed in the ramp-up phase to map the human brain at different levels of brain organisation

- 3.1.1. Anatomical and functional organisation
- 3.1.2. Connections between brain regions
- 3.1.3. Numbers and distributions of neurons and glia in different regions
- 3.1.4. Morphologies of neurons in different regions
- 3.1.5. Distribution of selected receptors in the human brain
- 3.1.6. The developing infant brain

Each task is broken down into the following stages:

- Method development
- Strategic data acquisition
- Integration of data

4.1 Anatomical and functional organisation

A top-down, functional parcellation of the whole human brain will be achieved using a massive fMRI protocol, combining major experimental categories designed across the world. Experimental design is being done in collaboration with SP3.

4.1.1 Method development

High-resolution imaging (T1 MRI, diffusion weighted-MRI and functional MRI [fMRI]) is being used, including a fast (multi-band) EPI acquisition scheme for fMRI that optimises spatial resolution (1.5mm with full brain coverage, repetition time=2s) while minimising the amount of spatial distortion. Specific techniques dedicated to learning a functional atlas from individual data have been developed.

4.1.2 Data acquisition

A reference cohort of 12 subjects (age range: 27-40 years) has been selected. Behavioural data have been collected to check that the subjects do not exhibit any neurological or psychiatric abnormality. The acquisition of genetic data will allow assessment of the absence of risk genotypes. Protocols have been developed (two pilots per protocol) and two anatomical/diffusion and eight functional protocols have been set up in a reference cohort of 12 subjects.

4.1.3 Integration of data

An analysis pipeline has been set up to obtain contrast-specific BOLD activation pre-processed in the MNI space. A functional brain atlas has been assembled from the pre-processed data, in which individual- and population-level delineation of functional areas is based on observed contrasts.

4.2 Connections between brain regions

Data on the major fibre tracts connecting the chosen subdivisions will be obtained using structural MRI, Diffusor Tensor Imaging (DTI) for living human brains and high-resolution Polarised Light Imaging (PLI) for *post mortem* brains.

4.2.1 Method development

Standard tractography-dedicated diffusion MRI will be applied for inference of an atlas of fibre bundles that is stable across a large population of subjects, including U-fibre bundles, which have been mapped for only very small portions of the brain. In a population of ten subjects, the distribution of axon diameters in the largest bundles will be assessed, using massive diffusion-time based MRI acquisitions. Software is being developed for mapping anatomical fibre tracts between functionally defined brain regions in standard space using diffusion MRI as a prerequisite for the integration of fibre information into cognitive models (SP3).

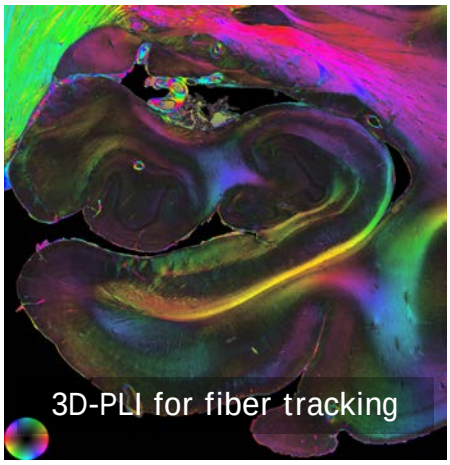

Figure 2: 3D-PLI for fibre tracking – region of interest in the human mesial temporal lobe. The different colours show direction of fibres according to the colour sphere. The resolution is 1.3 x 1.3 x 70 micrometres, and enables single fibres and small fibre bundles to be followed, and also reveals details of the cortical architecture.

The PLI-method is applied to demonstrate fibre architecture at micrometre resolution scale in adult *post mortem* brains, but also in rat brains as a model system (Figure 2). PLI data provide a gold standard for *in vivo*-based diffusion measurements. To link PLI with diffusion data, we have developed methods to extract PLI-based orientation distribution functions (ODFs) and have achieved diffusion-tensor–like descriptions, as used for *in vivo* imaging. In addition, we have streamlined the workflow for massive datasets. Finally, we have developed a basis for 3D-reconstruction of large PLI data sets from 450 histological sections in a rat brain. Here, it was necessary to develop a dedicated visualisation tool, which handles large data sets, in the range of several GBytes.

4.2.2 Data acquisition

- The inference relies on a dataset of 80 subjects previously acquired in the context of the CONNECT European consortium. Pilot acquisitions are performed during the

development of the axonal diameter dedicated MRI sequences. The second stage leads to the target dataset, relying on 5 acquisitions for each of the 10 subjects.

- For PLI, we have already measured 200 coronal sections of the human brain using the large-area polarimeter (spatial resolution 64 x 64 x 70 micrometres) and the polarising microscope (spatial resolution 1.3 x 1.3 x 70 micrometres). The average size of such sections was 10 x 10 cm. The measurement time was approximately 6h per section for resolutions in the micrometre range. In addition, we have digitised and analysed 450 sections of a rat brain using both systems (total measurement time: four months). On this basis, we have generated the first 3D-reconstructured PLI rat brain. These data provide pilot data in many respects (visualisation, tractography, segmentation, etc.), and they served as the basis for defining workflows for PLI analysis.

4.2.3 Integration of data

- The *in vivo* bundle atlas is mapped on each subject of the second dataset to identify the bundles. The bundle atlas will be aligned with the other SP2 atlases using the sulci-based DISCO alignment method. All atlases will be aligned with the MNI152 and the MNI single subject spaces, which will allow them to be represented in the Human Brain Atlas. Fibre tracts for specific functional systems will be delineated, quantitatively characterised and compared to functional connectivity (in collaboration with SP3, Cognitive Architecture) and made available within the MNI152 space. In addition, we have defined the necessary steps to align the data with the BigBrain data set as an alternative, high-resolution reference space.
- We have performed staining experiments to label cell bodies in histological sections used for PLI, and continue working on the quality of staining. Secondly, we were able to visualise cell bodies in unstained PLI sections (Figure 3). These data prove the feasibility of data analysis and processing in the ramp-up phase.

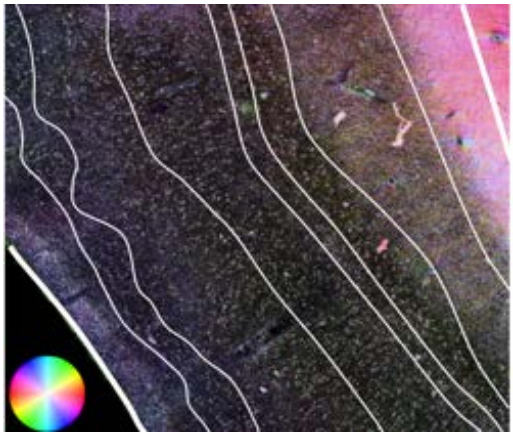

Figure 3: 3D PLI for fibre tracking - region of interest from the human entorhinal cortex. Image shows both the direction of fibres (colour-coded according to the colour sphere) and the localisation of cell bodies (light grey). Upper right corner: white matter, lower left – pial surface. Cortical layers are marked by white lines.

4.3 Numbers and distributions of neurons and glia in each brain region

Data on the numbers and distributions of neurons and glia in each brain region will be obtained using high-resolution cell counting under light microscopy. Together with information on the volume and dimensions of different brain regions, these data will make it possible to generate a cellular 3D representation of the human brain – a new reference brain on the microscopic level. It will provide the basis for extracting region-specific parameters, for example cortical surfaces, and cortical thickness. In defining brain regions, we will rely on the already existing cytoarchtitectonic probabilistic maps of the JuBrain atlas (https://www.jubrain.fz-juelich.de/apps/cytoviewer/cytoviewer-main.php). The reference brain will enable testing of hypotheses on optimal path lengths between interconnected cortical regions, or spatial organisation of genetic patterning, redefining the traditional neuro-anatomy maps such as those of Brodmann and von Economo.

4.3.1 Method development

- We will adapt and apply the methods developed in SP1 to analyse the numbers and distributions of excitatory and inhibitory neurons and glia to achieve comparable data for human and mouse brains. These include a new 3D segmentation algorithm, which allows neurons (stained with anti-NeuN antibody) to be automatically segmented in 3D from image stacks obtained by confocal microscopy, which provides accurate data concerning their spatial distribution and size. This method is now being adapted for use in the 3D segmentation of all cells in sections stained for DAPI (which labels the nuclei of both neurons and glia). Frozen sections from human brains, which were collected together with those processed for receptor autoradiography (Task 2.1.5.), will be processed. The cerebral cortex and other major brain regions will be characterised.
- The 3D segmentation algorithm generated by SP1's Task T 1.2.3 will be used, along with spatial statistical techniques that will be applied in collaboration with WP5.4: Predictive Neuroinformatics (more specifically, T5.4.2: Neuronal Structural Design and Predictions, Larrañaga, CIG-UPM). The aim is to characterise the density and principle patterns of spatial distribution of neurons and glia (DAPI nuclei of NeuN-positive and NeuN-negative neurons) in different human brain regions. In addition, human brain tissue is being processed (autopsy cases 2–3 h *post-mortem*) for immunocyto-chemistry, specifically to analyse two major subpopulations of GABAergic neurons, namely cells expressing the calcium-binding proteins parvalbumin and calretinin.
- We have further improved the workflow for 3D-reconstruction of a whole human brain data set based on cell-body stained histological sections, building on the successful work on the BigBrain data sets (Amunts et al., Science, 340(6139): 1472-1475). The new pipeline includes detailed logging of all processing steps, as well as parallel routines for 3D reconstruction. These new developments signifiantly decrease the reconstruction time for BigBrain 2, compared to that for BigBrain 1. We have provided the first 3D reconstruction with a resolution of 300 micrometres in-plane and 20 micrometres slice distance after 6 months (see Figure 4).

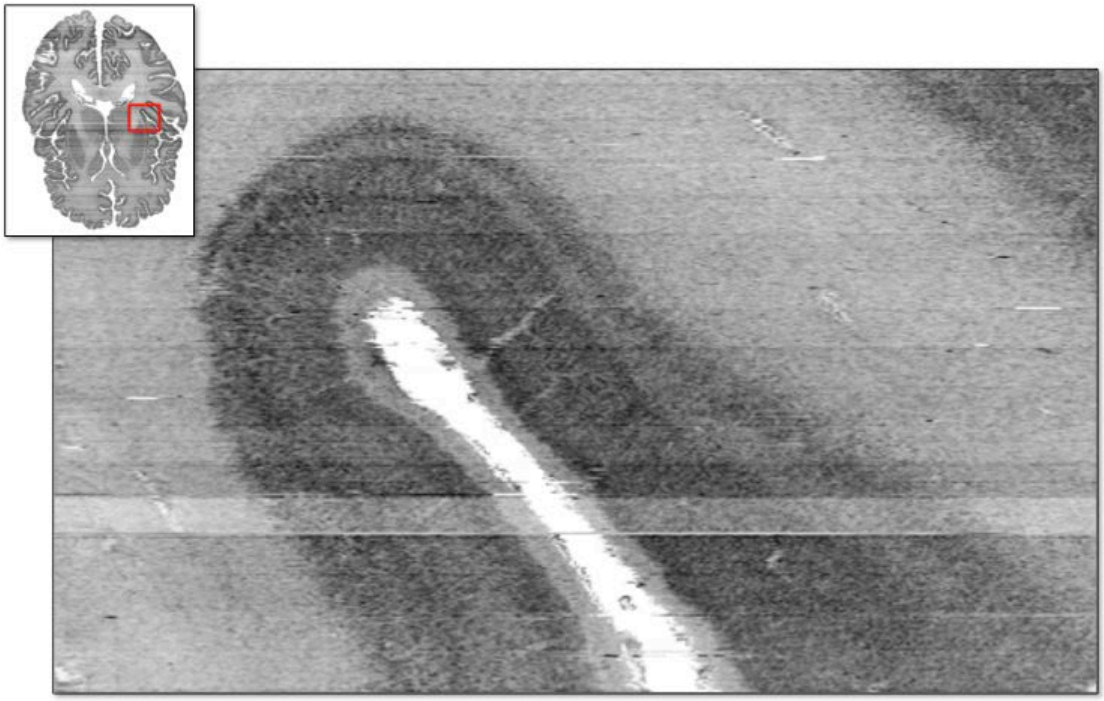

Figure 4: Virtual horizontal section of the second BigBrain data set in the horizontal plane (upper left). The brain was physically cut in the coronal plane, and then reconstructed in 3D. Note the high quality of details of cortical organisation in the virtual section at high magnification, enabling detailed analysis of cellular and sub-laminar structures

4.3.2 Data acquisition

- The second ultra-high resolution data set of a human brain has been obtained, digitised and fully reconstructed in 3D with a preliminary spatial resolution of 300 micrometres (20 micrometres in-plane). We are increasing the spatial resolution of the 3D reconstruction down to 20 micrometres isotropic.
- Different cortical regions are examined with respect to volume fraction of cell bodies, including the following Brodmann's cytoarchitectonic subdivisions: primary visual area 17, pre-motor area 6, associative lateral temporal areas 20 and 21, orbital frontal areas 11 and 12, dorsolateral frontal areas 9, 10 and 46, and anterior cingulate areas 24 and 32.
- Together with our partners from the McGill University in Montreal, we are segmenting the BigBrain data sets into grey and white matter.

4.3.3 Integration of data

We organised a meeting with our colleagues from the Allen Institute for Brain Research in Seattle in January 2014, and defined the workflow to co-register the BigBrain data set with the data sets of six human hemispheres mapped for gene expression data in Seattle. A second meeting on this topic took place in March 2014 in Juelich. The roadmap has been defined to integrate gene expression data available in six individual post-mortem brains with the BigBrain reference brain and data available in the single subject T1-weighted MNI reference brain. All brains have different spatial resolutions, data formats and spatial

orientation, which makes it necessary to calculate transformation between the different spaces.

4.4 Morphologies of selected human neurons

Data on the morphologies of selected human neurons will be obtained using single neuron and general Golgi labelling in *post mortem* tissue, followed by 3D morphological reconstructions of the labelled neurons. In addition, single neuron morphology data is available from brain slices of patients who undergo brain surgery by external researchers.

4.4.1 Method development

- Two strategies are currently being developed to obtain full 3D morphological reconstructions of different neuron types, as SP1 is doing in mice in SP1. Firstly, recordings are made in healthy neocortical and hippocampal tissue that has been surgically resected to gain access to deep epileptic foci or tumours. In conjunction with functional synaptic and neuronal physiology data, this will provide 3D neuronal reconstructions. Secondly, a collaboration has been established with the Netherlands Brain Bank in Amsterdam (http://www.brainbank.nl) that will provide, in the operational phase, the ability to record and label neurons in any region of a healthy human brain, within 2-3 hours (maximum 6 hours) of death. This will enable us to provide strategic data on full 3D morphological reconstructions, as well as single cell-type transcriptome (SCT) data from any neuron type in any area of the human brain.
- Ethical approval for this procedure has been obtained and the first recordings have been made.

4.4.2 Data acquisition

We have started building a reference data set that allows comparison of morphological data obtained from recordings in human brain slices with morphologies obtained from *post mortem* staining of human brains performed by T 2.1.2 and also with mouse brain data generated by SP1 (Strategic Mouse Brain Data).

4.4.3 Integration of data

Interaction with research groups worldwide that are recording and reconstructing human neurons from brain parts excised during neurosurgery has started. This co-ordination will make it possible to standardise the methods used to reconstruct neurons and to match human to mouse neurons - in terms of their physiological properties, maximising the value of reconstructed cells and identifying candidates for reconstruction (human cells that match well-established cell-types in the mouse brain).

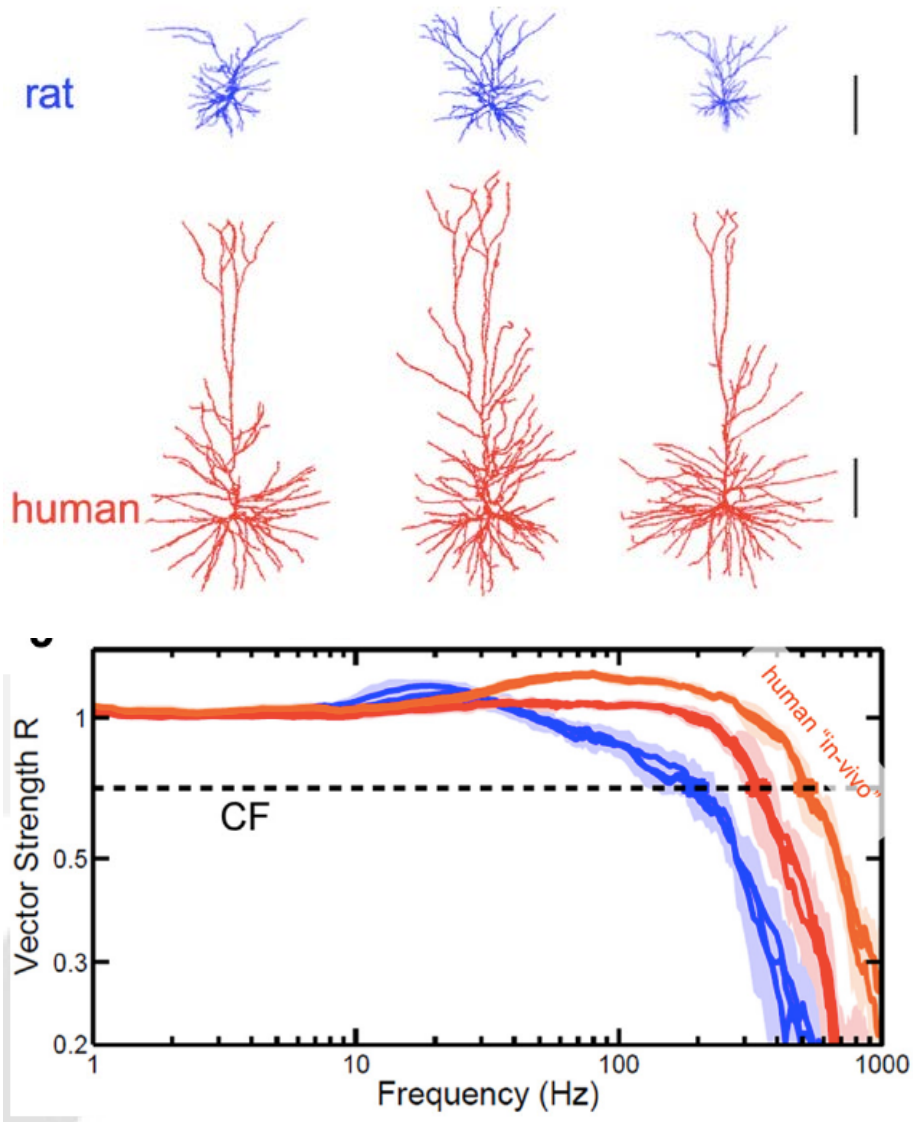

Figure 5: Relation between size of dendritic tree and axonal spikes in mouse and human. The large dendritic tree of human layer 2/3 neurons (red neurons) allows to encode input modulations via axonal spikes at much higher frequencies (bottom plot, c) than layer 2/3 neurons of rats (blue neurons), which have much smaller dendritic trees. Scale bar, 200 um; Bottom ©, Vector strength in human (red) and rats (blue) as a function of the frequency of input modulations (Eyal, Mansvelder, De Kock, and Segev (2014), Journal of Neuroscience, in press).

4.5 Distribution of selected receptors in the human brain

Data on the distribution of selected receptors in the human brain will be obtained using quantitative radio- and immuno-histochemical staining. Absolute concentrations of at least 20 receptors for classical neurotransmitters with sub-laminar spatial resolution will be provided in the ramp-up phase. In addition, data needed to allow spatial referencing to

other data modalities will be generated, including cytoarchitecture, connectivity and *in vivo* neuroimaging data.

4.5.1 Method development

Transmitter receptors are measured by quantitative *in vitro* receptor autoradiography. The method will be developed and established in the laboratory, based on the experience in animal brains. It includes:

- Deep freezing of human hemispheres
- Preparation of 20 micron thin cryostate sections,
- Incubation of sections in solutions containing tritium-labelled molecules, which bind with high affinity and specificity to the respective receptor type
- Exposition of the sections against tritium-sensitive films
- Scaling of the grey value distribution in the autoradiographs by standard probes of known radioactivity
- Calculation of the regional (mean) and laminar receptor densities in fmol/mg protein; colour coding of the digitised autoradiographs.

A standardised pipeline for all these steps is being established.

4.5.2 Data acquisition

- Absolute receptor densities will be measured in cytoarchitectonically and/or functionally defined cortical regions of the human brain. Receptor densities are measured both as mean density averaged over all cortical layers and as profiles from the cortical surface to the cortex/white matter border. This latter procedure provides profiles indicating the layers specific increase or decrease of the receptor density throughout the cortical width.
- Receptor data of six human hemispheres will be provided.
  - The densities of 23 different receptor binding sites will be measured:
  - Glutamate receptors AMPA, NMDA, kainate, and metabotropic GluR2/3
  - GABA receptors, $GABA_A$ (both agonistic and antagonistic binding sites), $GABA_B$, and benozodiazepine-binding sites of $GABA_A$
  - Acetylcholine receptors, muscarinic M_1, M_2 (both agonistic and antagonistic binding sites), M_3, and nicotinic
  - Dopamine receptors D_1, D_2 and D_4; serotonin receptors 5-HT_{1A} and 5-HT_{2A}
  - Noradrenaline receptors α_1 and α_2 (both agonistic and antagonistic binding sites); adenosine receptors A_1 and A_{2A}.
- During the ramp-up phase, these binding sites will be measured in primary sensory, motor and selected multimodal areas (particularly language-related brain regions) of the cerebral cortex in six human hemispheres.

4.5.3 Integration of data

- Since the receptor densities are measured in cytoarchitectonically and/or functionally distinct cortical regions, the data set for each receptor and the multi-receptor fingerprint can be assigned to the corresponding area in the cytoarchitectonic brain atlas. This provides a first step towards a multimodal human brain atlas, comprising regional and laminar receptor density profiles.
- After this first step, a 3D-reconstruction of the continuous receptor distribution throughout the whole forebrain of six hemispheres will be performed.
- During the ramp-up phase, 3D-reconstruction of the regional and laminar distribution of the muscarinic M_2 receptors will be achieved, since this receptor shows the most distinct regional differences, and is a major modulatory receptor in the cerebral cortex.
- The warping of these six hemispheres containing the M_2 receptor distribution to the reference brain of the atlas will provide information about the inter-subject variability of receptor expression throughout the human cortex.

4.6 The Developing Human Brain

This workstream will provide infant-dedicated tools and data to obtain a better description of the morphological development of the human brain from 29 weeks gestational age (preterm) to two years post-term, and of the correlation between morphological and functional development, in order to understand human learning capacities.

4.6.1 Method development

- An atlas of fibre bundles in a population of infants (6 to 22 weeks old) based on diffusion MRI and tractography will be implemented.
- The cortical parcellation defined in an infant template brain (Kabdebon et al, in revision) will then be propagated to the same population of infants with the sulci-based DISCO alignment method (see below).
- The maturation of these infant cortical regions will be provided using quantitative T1 and T2 mappings, based on elastic registration to anatomical images.
- Finally the tools will be validated automatically by definition of fibre bundles and cortical regions in another population of infants (~4 weeks old).

4.6.2 Data acquisition

- Data have been acquired in 29 healthy infants, born at term and aged between 6 to 22 weeks old at MRI. T2w anatomical images were obtained for 27 of them, diffusion images for 22, and quantitative T1 and T2 mappings for 17. These data are being used to implement atlases of fibre bundles and cortical regions.
- Data from 13 young adults have been acquired with the same protocol. A new set of infant data will be acquired in the year ahead to validate our tools.

4.6.3 Integration of data

- The bundle and cortical atlases are being mapped for each subject in the second infant data set.
- These atlases will further be aligned with the adult data set using the sulci-based DISCO alignment method.
- All atlases will be aligned with the MNI 152 and the MNI single subject spaces.

5. Work Process: data aggregation, analysis and dissemination

This chapter provides information on how the process of data aggregation, analysis and dissemination is organised. It concerns standardisation of techniques, the selection of appropriate tools and the management of data. Furthermore it will describe how human brain data are deposited in the HBP Atlas and Brainpedia.

5.1 Standardisation of techniques and tools

- Existing, standard brain alignment methods are not optimal in the context of the heterogeneous atlases generated by SP2. Therefore, several algorithmic tunings of a constraint-based differomorphic alignment method called DISCO have to be performed, according to several scenarios relevant for SP2:
  - Sulcus-based alignment of several subjects,
  - Sulcus based alignment of adult and infant atlases,
  - Sulcus based alignment of *post mortem* versus *in vivo* atlases.
- DISCO is also being adapted to align SP2 atlases with SP3 data.
- After the ramp-up phase, DISCO shall be provided as a service to the community through the HBP Unified Portal.
- The tools developed will be applied to the different atlases provided by other Tasks to improve their alignment.
- They will also be applied to improve the atlas alignment with single subject and ICBM 152 MNI space.
- Infrastructure available: Neurospin 500 core cluster.

5.1.1 Data management:

- Software secure versioning is being achieved with subversions.
- Reverse engineering is being tested to convert the prototype into a user-friendly software and the first successful experiments have been performed.
- The next targets are being tackled in parallel.
- High-resolution PLI analysis requires specific steps related to the 3D-reconstruction, and for generating Fibre Orientation Maps. The workflow for generating such data has

been developed (Figure 6). Considering the large sample size for this data (up to 700 TByte for an individual data set at micrometre-resolution), several steps have to be performed in parallel, and run on Juelich Supercomputers in collaboration with SP7.

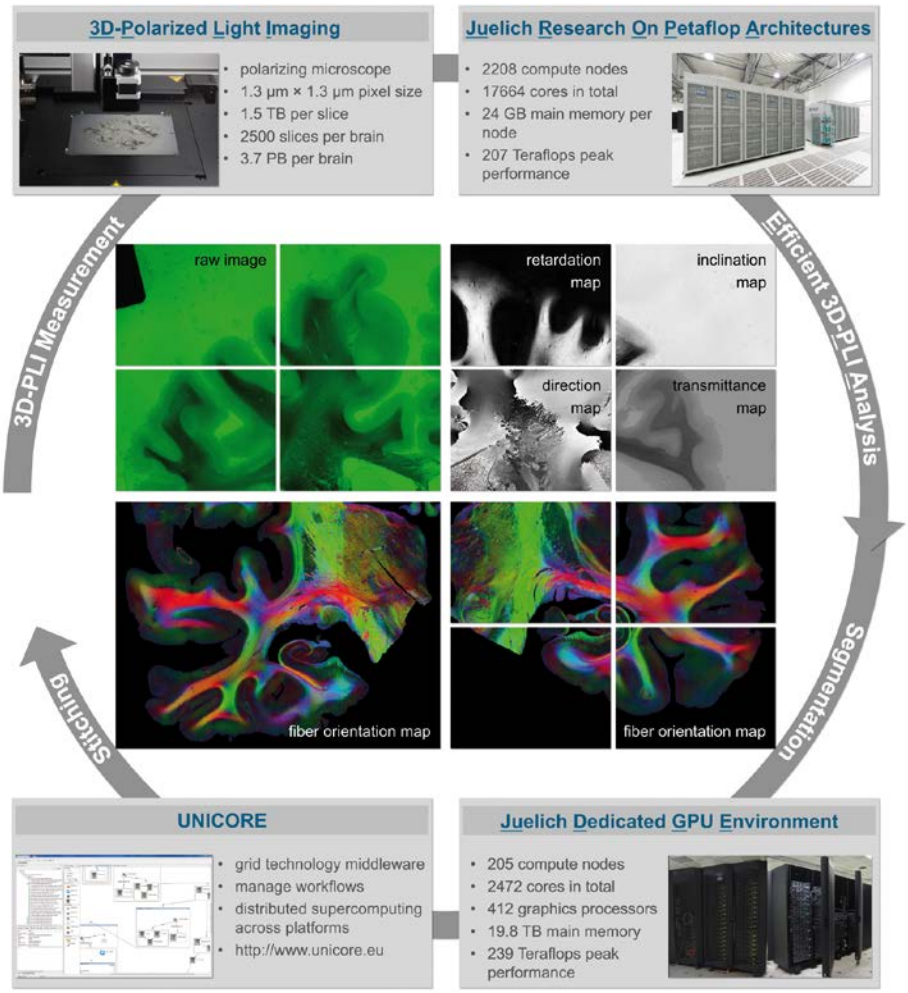

Figure 6: General workflow from PLI-data sets

5.2 Deposition of Human Brain Data in the HBP Atlas and Brainpedia

This task is done in close collaboration with SP5 (Neuroinformatics Platform). For a detailed description of the supported reference spaces, see the SP5 Deliverable D 5.8.1.

5.2.1 Deposition of data characterising the anatomical and functional segregation

The data (images and metadata) will be transferred to the HBP Neuroinformatics Platform (SP5). A repository will be set up on an IDS data server managed by the French INCF Node. Prototype *in vivo* datasets will be used to finalise the IT solution. A dataflow dedicated to HBP acquisitions will then be defined.

5.2.2 Deposition of data characterising the connections

The bundle atlas will provide:

7) A set of 3D bundles for simple visualisation
8) A set of probability maps describing the localisation variability of each bundle in the MNI spaces
9) The largest bundle will come with a description of the axon diameter distributions and across subject variability
10) Major fibre tracts for at least two functional systems will be mapped, quantitatively characterised and deposited in the Brain Atlas during the ramp-up phase.

5.2.3 Deposition of post mortem data on the cellular & receptor architecture and neuronal morphologies

- The data from the first BigBrain data set have been transferred to the HBP atlas site. Since we had not yet established a mechanism to transfer such large data sets (1 Tbyte) via Internet, they have to be shipped for the time being. In parallel, mechanisms are being worked out for future electronic transfer in the ramp-up phase. The volume of data to be processed is challenging, and requires new approaches to data handling. The BigBrain data set will provide a standard reference space for multimodal data on brain organisation, and will be made available to the neuroscientific community.
- The first morphologies have been fed into SP5's Neuroinformatics Platform (Segev, Telefonte).
- With SP5, we are developing workflows and identifying metadata necessary for the integration of receptor-architectonic data into the HBP atlas, as a prerequisite of the multimodal human brain atlas.

5.2.4 Deposition of data from infant brains

- The infant bundle atlas is being provided as a set of 3D bundles for simple visualisation, as well as a set of probability maps describing the localisation variability of each bundle in the MNI spaces.
- An infant template parcelled in 94 cortical regions is ready for provision. We have reported the anatomical variability of six main cortical sulci and of external landmarks (10-20 sensors placement) in this template.

6. The Measuring of Progress by Key Performance Indicators

The progress of work will be measured using the Key Performance Indicators as described in the HBP Description of Work, namely categorical stage indicators, aggregate stage indicators, and numerical indicators.

6.1 Categorical Stage Indicators

Categorical Stage indicators in SP2 are following the different levels of brain organisation and the management of data. They are listed in Table 1:

Table 1: Categorical Stage Indicators for SP2

Task	Activity	Month				
		6	12	18	24	30
2.1.1	Evaluation of local committee for IRB approval completed	x				
	State of the art of distortion correction techniques for the data acquisition parameters have been reviewed (analysis of the HCP data) and pilot acquisitions have been run		x			
	Functional paradigms to be used have been tested and the release and piloting based on these protocols have been started		x			
	The recruitment of subjects has started after the IRB approval being accomplished			x		
	The informatics methods and tools will be evaluated and the analysis pipeline installed					x
	Images available to feed the atlas that best quantify the richness of the database is being measured	x	x	x	x	x
2.1.2	Fibre Tract atlas method: Using DISCO to align subjects before inference		x			
	Methods for diffusion-based data completed		x			
	Methods for existing tracts scans completed		x			
	First axonal calibration and diffusion-based fibre scans			x		
	Axon data for adult tracts				x	

Task	Activity	Month				
		6	12	18	24	30
2.1.2 cont'd	DTI-PLI alignment method evaluated and running					x
	T1 and T2 relaxation time for major tracts					x
	Provide an off-line useable software for the integration of post-mortem cytoarchitecture and functional imaging		x			
	Software available for general use		x			
	Initial method for identifying core fibres connecting functional regions implemented		x			
	Method for identifying core fibre connections is optimised and tested in a first set of regions			x		
	Provide a description of major adult tracts					x
2.1.3	Establishment of automated methods to generate maps of neurons and glia:					
	Segmentation algorithm development	x				
	Selection of spatial statistical techniques	x				
	Implementation and validation of methods		x			
	Applications of methods on the brain regions: primary visual area 17, secondary motor area 6, associative lateral temporal areas 20 and 21.			x		
	Definition of numbers of neurons and glia in the above regions			x		
	Applications of methods on the brain regions: orbital frontal areas 11 and 12, dorsolateral frontal areas 9, 10 and 46, and anterior limbic cingulate areas 24 and 32.				x	
	Definition of numbers of neurons and glia in the above regions				x	

Task	Activity	Month				
		6	12	18	24	30
2.1.4	Ethical approval and first recordings in peri-mortem brain. First contact with groups worldwide that record from healthy adult human neurons.		x			
	Building reference data set on temporal cortex pyramidal neuron morphologies from peri-mortem brain.			x		
	Reference data set on temporal cortex pyramidal neuron morphologies from peri-mortem brain complete				x	
	First reference morphologies data sets of temporal cortical pyramidal neurons compared among three methods (see Timeline).					x
	A research agenda being designed in collaboration with groups world-wide for strategy of obtaining strategic 3D morphologies and SCT data from human neurons in different brain areas in the HBP operational phase					x
2.1.5	Provide methods for the measurement of mean receptor density and laminar receptor profiles		x			
	Provide mean receptor density and laminar profiles of the muscarinic M_2 receptor in primary sensory and motor cortical areas in human brains			x		
	Provide 3D-reconstrucion of 2 human hemispheres				x	
	Provide mean receptor densities and laminar profiles for all 23 receptor binding sites of primary motor and sensory (visual, auditory, somatosensory) cortical areas as well as selected language related areas in 6 human hemispheres					x
	Provide the receptor distribution map warped to the reference brain of the human brain atlas					x

Task	Activity	Month				
		6	12	18	24	30
2.1.6	Definition of individual bundles and the cortical template from infant and adult data	x				
	Maturation of 8 major white matter infant tracts		x			
	Maturation of 94 regions defined on our infant template in relation with Tzourio-Mazoyer et al-atlas in adults					x
	DISCO alignment method and elastic registration tested in infants		x			
	Atlas implementation being performed		x			
	Atlas propagation and integration tests completed					x

4.2 Aggregate Stage Indicators

Aggregate stage indicators for method development used in SP2 have been defined by identifying key elements from the activities stated previously in Table 1. They are listed in Table 2 along the Tasks and the stage, namely research, development and deployment.

Table 2: Aggregate stage indicators to measure the progress of work

Goal	Activity	Stage	Month				
			6	12	18	24	30
Generating data on the anatomical and functional organisation of the human brain	Selection of a reference cohort of 12 subjects [age range 27-40 yrs] and acquisition of behavioural data to check that the subjects do not exhibit any neurological or psychiatric abnormality	Research Development Deployment			100		
	Setting up the protocols to be used (2 pilots per protocol)	Research Development Deployment		25	50	75	100
	Acquisition of 2 anatomical/diffusion and 8 functional protocols in a reference cohort of 12 subjects	Research Development Deployment				50	100
Develop methods to study the connections between brain regions	Off-line useable software for the integration of post-mortem cyto-architecture and functional imaging (E)	Research Development Deployment	100 50 0	 100 100			
	Method for identifying core fibre connections to finally describe major adult tracts (E)	Research Development Deployment		50 0 0	100 50 0	 100 50	 100

Goal	Activity	Stage	Month				
			6	12	18	24	30
Measure the number and distribution of neocortical cells in the human brain	Establishment of automated methods to generate maps of neurons and glia (Confocal microscopy)	Research Development Deployment	100 50 0	 100 0	 25-50	 50-100	
	7,676 3D ultra-high resolution scans ready for mapping	Research Development Deployment	100 100 10	 50	 100		
	Software being developed to analyse 20µm thin 3D scans	Research Development Deployment	100 50 0	 100 0	 25-50	 50-100	
Identify the morphologies of human neurons in different brain regions	Methods for recordings in peri-mortem brain	Research Development Deployment	100 25 0	 50 25	 75 50	 100 75	 100
Measure the distribution of receptors in the human cerebral cortex	Method for measurement of mean receptor densities and laminar receptor profiles	Research Development Deployment	100 50 0	 100 100			
	M_2 receptor measurements in human hemispheres	Research Development Deployment	100 50 0	 100 50	 100		
	3D-reconstruction of 2 human hemispheres based on receptor imaging	Research Development Deployment	20 0 0	70 50 20	100 100 70	 100	

Goal	Activity	Stage	Month				
			6	12	18	24	30
Measure the distribution of receptors in the human cerebral cortex (cont'd)	Measurements of mean densities and laminar profiles of 23 receptors in 6 human hemispheres	Research Development Deployment	0 0 0	20 20 0	60 60 20	100 100 60	 100
	Receptor distribution map warped to the reference brain of the human brain atlas	Research Development Deployment	20 0 0	40 20 0	60 60 0	80 80 0	100 100 100
Study the Developing Human Brain	Maturation of white/grey matter regions reported on infant template in relation with Tzourio-Mazoyer et al-atlas in adults (2002)	Research Development Deployment	20	40	60	80	100
	Measurements of 20 infant brains to infer the atlases	Research Development Deployment	100	100	100	100	100
	Measurements of 20 infant brains to test the atlases	Research Development Deployment			50	80	100

6.2 Numerical Stage Indicators

The progress of data generation is monitored by numerical indicators according to the specific kind of data that is generated in each task. Numerical indicators and target values for Month 6, 12, 18, 24 and 30 are displayed in Table 3:

Table 3: Numerical Stage Indicators

Task	Indicator	Month (Target)				
		6	12	18	24	30
2.1.1	Number of images of functional contrast of interest available for atlas inference				240	480
2.1.2	Number of subjects used to infer the atlas	12				
	Number of reproducible bundles in the atlas	20				
	Number of axon diameter dedicated acquisition sessions	10				
	Number of atlas bundles with estimation of axon diameters	1				
	Number of tracts mapped in the course of the pipeline development			10		
	Number of tracts mapped and quantitatively characterised					50
2.1.3	Number of cellular maps in different brain regions (11)	0	0	4-6	11	
	Number of maps of individual identified neurons	0	0	50	100	
	Number of high-resolution scans	130	3,000	7,676		
	Number of 200 strongly damaged 3D 20um thin scans reconstructed	2	150	200		
	Number of 500-1000 lightly damaged 3D 20µm thin scans reconstructed	10	200	500-1000		

Task	Indicator	Month (Target)				
		6	12	18	24	30
2.1.4	Number of neurons in a reference data set of temporal cortex pyramidal neurons from peri-mortem recordings		3	15	25	
	Number of neurons in data sets of temporal cortex pyramidal neurons from 3 methods compared					75
2.1.5	No of hemispheres sectioned, incubated and exposed to film	2	6			
	No of autoradiographs digitised	150	300	5,000	10,000	21,000
	No of mean receptor densities measured	0	0	12	150	276
	No of laminar profiles of receptor densities measured	0	0	36	108	2,484
2.1.6	Number of infants tested to infer the atlases	20				
	Number of infants tested for the atlases					20
	Number of T2w anatomical images in infants	27				
	Number of diffusion images in infants	22				
	Number of T1 and T2 mappings in infants	17				
	Number of young adults tested	13				

5. Conclusions

This document shows that strategic brain mapping is a vital part of the HBP. It shows how SP2's data generation work is organised around the different levels of brain organisation, with a focus in the ramp-up phase on developing methods for mapping the human brain. Interactions with other SPs in HBP are also described, as are progress indicators and target values. The division of work into different stages (method development, strategic data acquisition and integration, plus deposition of the data in the Brain Atlas) following an exact and complex schedule is illustrated.

Thus, SP2 is well placed to fulfil its role in the ramp-up phase and generate strategic multi-level information about the structure of the human brain, using high-resolution analysis of post-mortem brains and non-invasive neuroimaging of the living human brain to link structural organisation principles with cognitive functions.

These data will play a critical role in the development of the human brain models and modelling tools that the Brain Simulation Platform will make available in the operational phase. These models will provide a framework that will allow systematic integration of new human brain data generated anywhere in the world.

The generation of strategic human brain data by SP2, together with strategic mouse data from SP1 and data about cognitive architectures from SP3, will drive the development of neuroinformatics models and inspire the development of hardware to analyse large amount of data (Big Data), as well as development of new medical diagnostic tools and therapies.