EBRAINS brain atlas services:

Exploring and analysing the brain in its different facets

Status at M8

(D4.2 – SGA3)

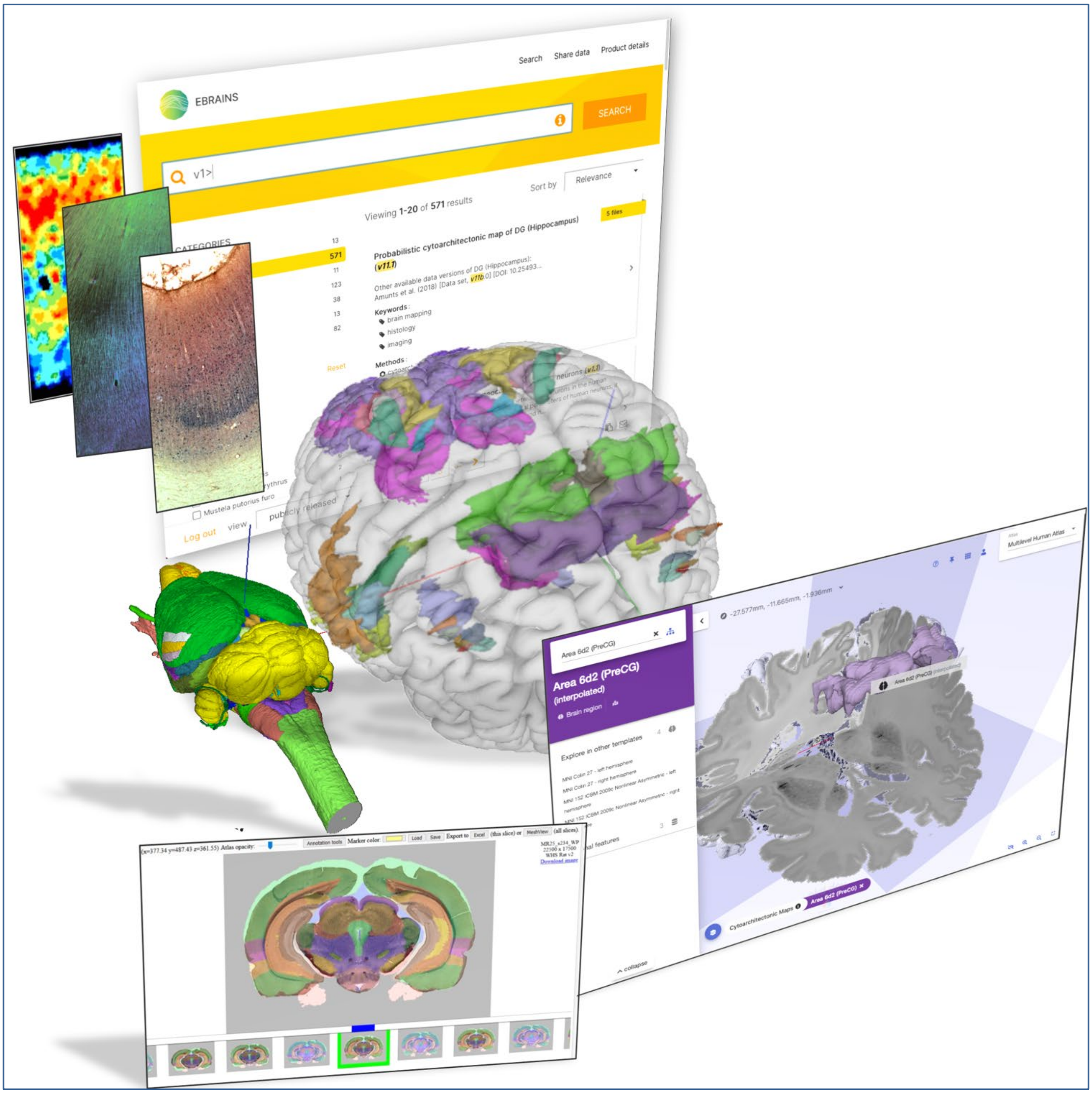

Figure 1: EBRAINS atlas services allow to explore and analyse the brain in its different facets.

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T7.4 QC Review:	Martin TELEFONT, EPFL (P1) - sci/tech QC Annemieke MICHELS, EPFL (P1) - editorial QC
Description in GA:	Updated brain atlases including full coverage of cytoarchitectonic probabilistic maps and first microscopic resolution maps for human.

Abstract:	EBRAINS provides access to reference atlases for the human, rat and mouse brain. Guided via the EBRAINS web portal, they can be explored using the 3D Interactive Atlas Viewer, as well as a 2D viewer for serial section images and, coming soon to EBRAINS, a Rest API and Python Client for structured programmatic access. Atlas services link to the EBRAINS Knowledge Graph for storing maps as curated datasets and giving access to a growing set of neuroscience datasets as multimodal data features of brain regions. To facilitate continuous integration of data to atlases, online tools are in place for spatial registration of common data types. Finally, a selection of workflows enable to analyse data in the spatial context defined by the reference atlases. This document describes the status of EBRAINS Brain Atlases Services in November 2020 (month 8) of SGA3 and highlights recent developments since April 2020.
Keywords:	Atlases, Spatial data integration, Data analysis tools
Target Users/Readers:	computational neuroscience community, computer scientists, Consortium members, funders, general public, neuroimaging community, neuroinformaticians, neuroscientific community, neuroscientists, Platform users, researchers, scientific community, students

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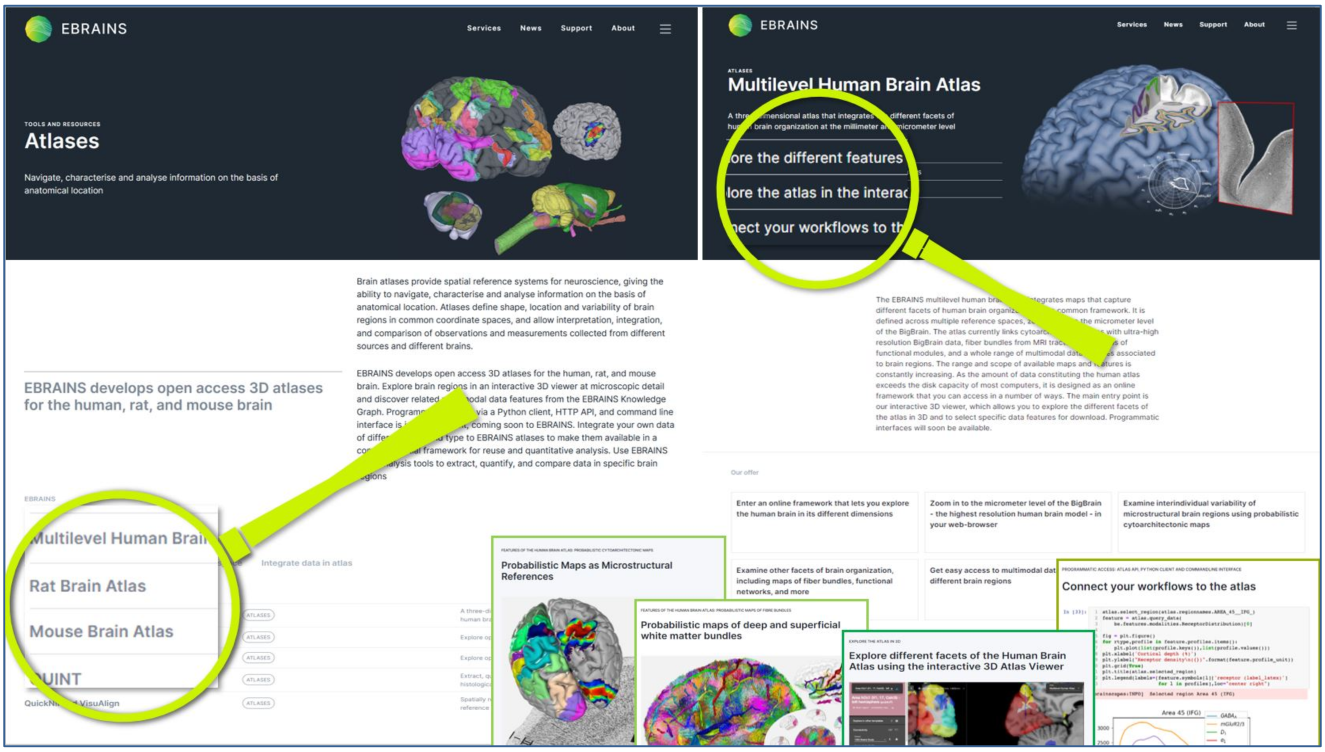
History of Changes made to this Deliverable (post Submission)

Date	Change Requested / Change Made / Other Action
21.12.2020	Deliverable submitted to EC
23.03.2021	Resubmission with specified changes requested in Review Report: • An analysis of known problems, delays and risks is missing and should be added
21.04.2021	Revised draft sent by WP to PCO. Main changes made, with indication where each change was made: 1. Analysis of known problems, delays and risks added (see Annex) Minor changes made: 1. Fixed URL to JuGEx documentation (p.20) 2. Update of JuGEx Sceenshots to current release version (p.20, Figure 18) 3. Fixed URLs of the hippocampus dataset cards (HC-xxx) and added version numbers (p.21) 4. Removed preliminary Python library name, as it will be changed in the next release (p.25, p.28)
23.04.2021	Revised version resubmitted to EC by PCO via SyGMa

1. Introduction

EBRAINS provides access to brain atlases for three different species: A multilevel atlas of the human brain, the Waxholm space atlas of the Sprague Dawley rat, and the Allen Mouse Brain Atlas. The human and rat atlases are developed in the Human Brain Project (HBP). The HBP is also developing a basic atlas of the monkey brain which will be included in the future (see 4.1). The general entry point for EBRAINS Atlases services is the EBRAINS web portal at https://ebrains.eu, which provides basic information about the brain atlases and the most important tools to access and work with them (Figure 2).



Navigate, characterise and analyse information on the basis of anatomical location

EBRAINS develops open access 3D atlases for the human, rat, and mouse brain

Brain atlases provide spatial reference systems for neuroscience, giving the ability to navigate, characterise and analyse information on the basis of anatomical location. Atlases define shape, location and variability of brain regions in common coordinate spaces, and allow interpretation, integration, and comparison of observations and measurements collected from different sources and different brains.

EBRAINS develops open access 3D atlases for the human, rat, and mouse brain. Explore brain regions in an interactive 3D viewer at microscopic detail and discover related odal data features from the EBRAINS Knowledge Graph. Program via a Python client, HTTP API, and command line interface is , coming soon to EBRAINS. Integrate your own data of diffe type to EBRAINS atlases to make them available in a co al framework for reuse and quantitative analysis. Use EBRAINS alysis tools to extract, quantify, and compare data in specific brain gions

A thre mensional atlas that integrates different facets of hu brain organization at the millimeter a micrometer level

The EBRAINS multilevel human bra tegrates maps that capture different facets of human brain organiz common framework. It is defined across multiple reference spaces, the micrometer level of the BigBrain. The atlas currently links cytoarc with ultra-high resolution BigBrain data, fiber bundles from MRI trac s of functional modules, and a whole range of multimodal dat s associated to brain regions. The range and scope of available maps and atures is constantly increasing. As the amount of data constituting the human atlas exceeds the disk capacity of most computers, it is designed as an online framework that you can access in a number of ways. The main entry point is our interactive 3D viewer, which allows you to explore the different facets of the atlas in 3D and to select specific data features for download. Programmatic interfaces will soon be available.

Enter an online framework that lets you explore the human brain in its different dimensions

Zoom in to the micrometer level of the BigBrain - the highest resolution human brain model - in your web-browser

Examine interindividual variability of microstructural brain regions using probabilistic cytoarchitectonic maps

Examine other facets of brain organization, including maps of fiber bundles, functional networks, and more

Get easy access to multimodal dat different brain regions

Figure 2: An impression of the atlas pages on the EBRAINS web portal.

Today, the main interface to EBRAINS atlases is the 3D Interactive Atlas Viewer, which can be freely accessed using most modern web browsers at https://atlases.ebrains.eu/viewer (Figure 3). It allows to explore the maps in 3D at different spatial scales and discover many data features linked to brain regions (see 2.2.1). Additionally, a 2D viewer for browsing images for serial sections in atlas space with sliced atlas region delineations is available. EBRAINS is also developing a dedicated Rest API and Python Client for structured programmatic access (see 2.2.4) which is currently available as a prototype.

With few exceptions, all maps and data features offered by the EBRAINS Atlases services are curated and shared as datasets via the EBRAINS Knowledge Graph[1] (see 2.2.3). With EBRAINS data curation as a part of the atlas development workflow, it is ensured that the atlases follow the practices of FAIR data and comply with the metadata standards promoted by EBRAINS. An OWL ontology model for the reference atlases is in development, and a compatible metadata model (openMINDS_SANDS) was released in November 2020.

A key feature of EBRAINS Atlases services is the growing set of neuroscience datasets that are linked to specific brain regions and exposed as multimodal data features (Figure 3). Most of these data features are datasets in the EBRAINS Knowledge Graph that have undergone spatial registration (anchoring) in the curation process, which assigns anatomical location to the data. Additional interfaces are available for region-agnostic access to selected external data repositories like the Allen Human Brain Atlas.

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[1] https://kg.ebrains.eu/search/?facet_type%5b0%5d=Dataset

To ensure ongoing integration of datasets to EBRAINS atlases, EBRAINS Atlases services offer online tools for common cases of anchoring datasets to atlases, including spatial alignment of 2D images, 3D partial volumes, and 3D geometries (see 2.4).

Finally, EBRAINS Atlases services include selected workflows for analysing data in a spatial context defined by the reference atlases. These cover extraction of image features in specific atlas regions, systematic and reproducible comparison of regional features, and projection of atlas contents to user data. The services are designed to be extended with more analysis tools and workflows in the future (see 4.4).

This document describes the status of EBRAINS Atlases services in November 2020 (month 8) of SGA3 and highlights some recent developments since April 2020.

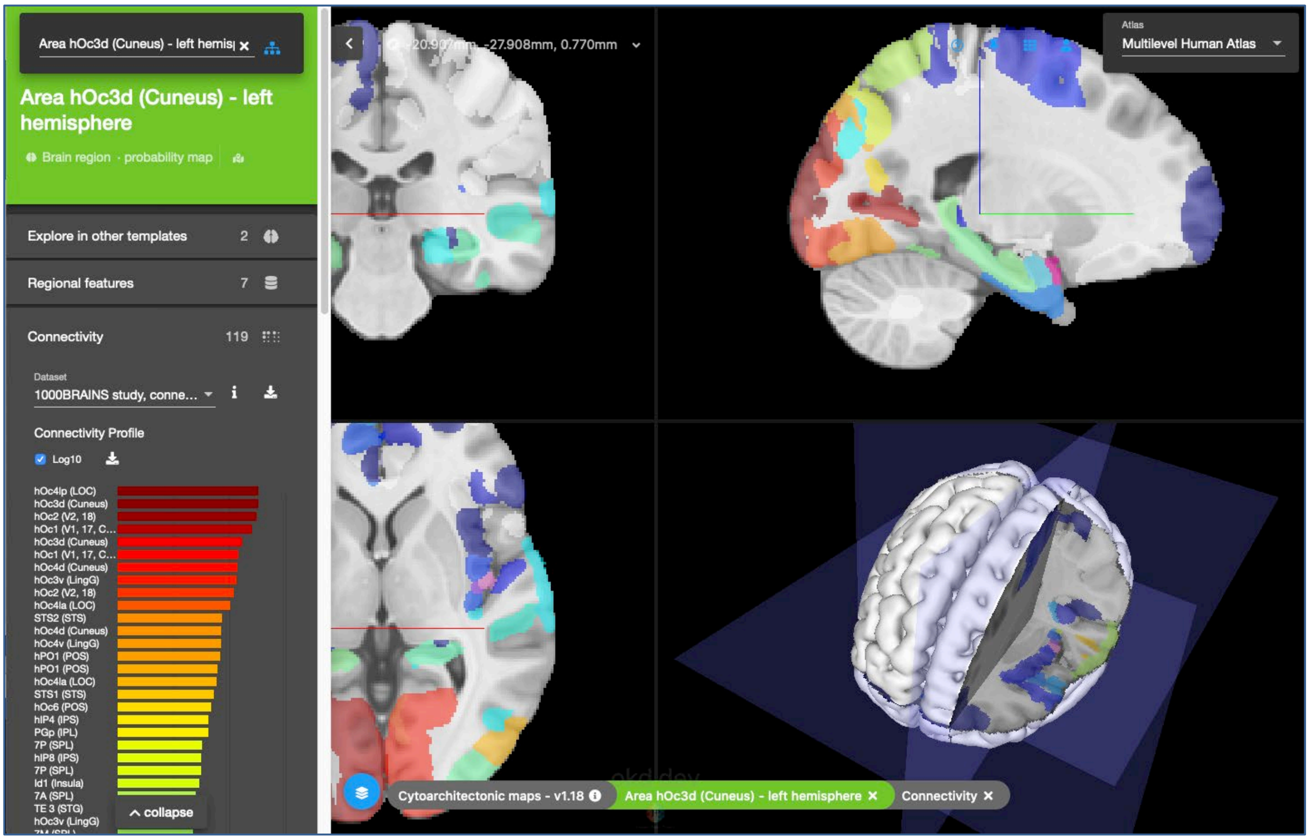


Figure 3: EBRAINS 3D Interactive Atlas Viewer.

The EBRAINS 3D Interactive Atlas Viewer showing the Julich-Brain cytoarchitectonic maps in MNI space, browsing connection strengths from area hOc3d to other areas extracted from diffusion imaging in a large cohort. This view is available at https://atlases.ebrains.eu/viewer/saneUrl/icbm_hOc3d .

2. Current status of EBRAINS atlas services

2.1 Atlases of different species

2.1.1 Multilevel Human Atlas

The EBRAINS multilevel human brain atlas goes beyond the typical structure of a single labelled brain volume with associated region names. Aiming to capture the many facets of human brain organisation in an integrated framework, it combines different complementary maps across multiple template spaces, and links them with diverse regional data features.

The framework is defined across multiple reference spaces (Figure 4). It supports the MNI Colin27 and ICBM 2009c nonlinear asymmetric spaces to reflect the macroanatomical scale, which are common in functional imaging and whole-brain connectivity studies. To represent brain organisation at the cellular level, it combines those with the microscopic resolution BigBrain model (Amunts K et al., 2013). Coordinates between these spaces are related by nonlinear diffeomorphic transformations (Lebenberg J et al., 2018) (P1391), which are implemented into a spatial transformation module. This allows EBRAINS atlas services to use approximate spatial links between the different template spaces.

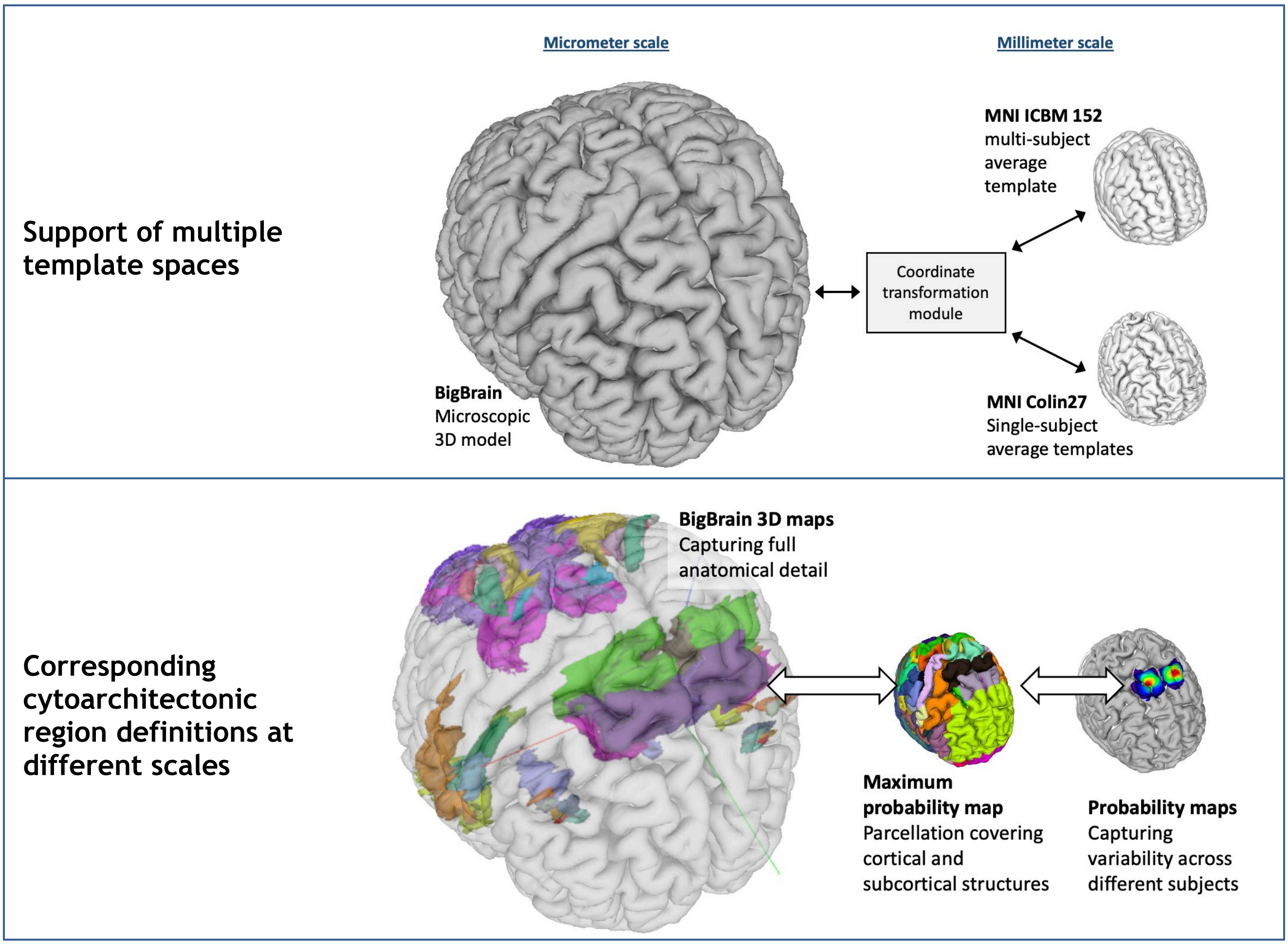


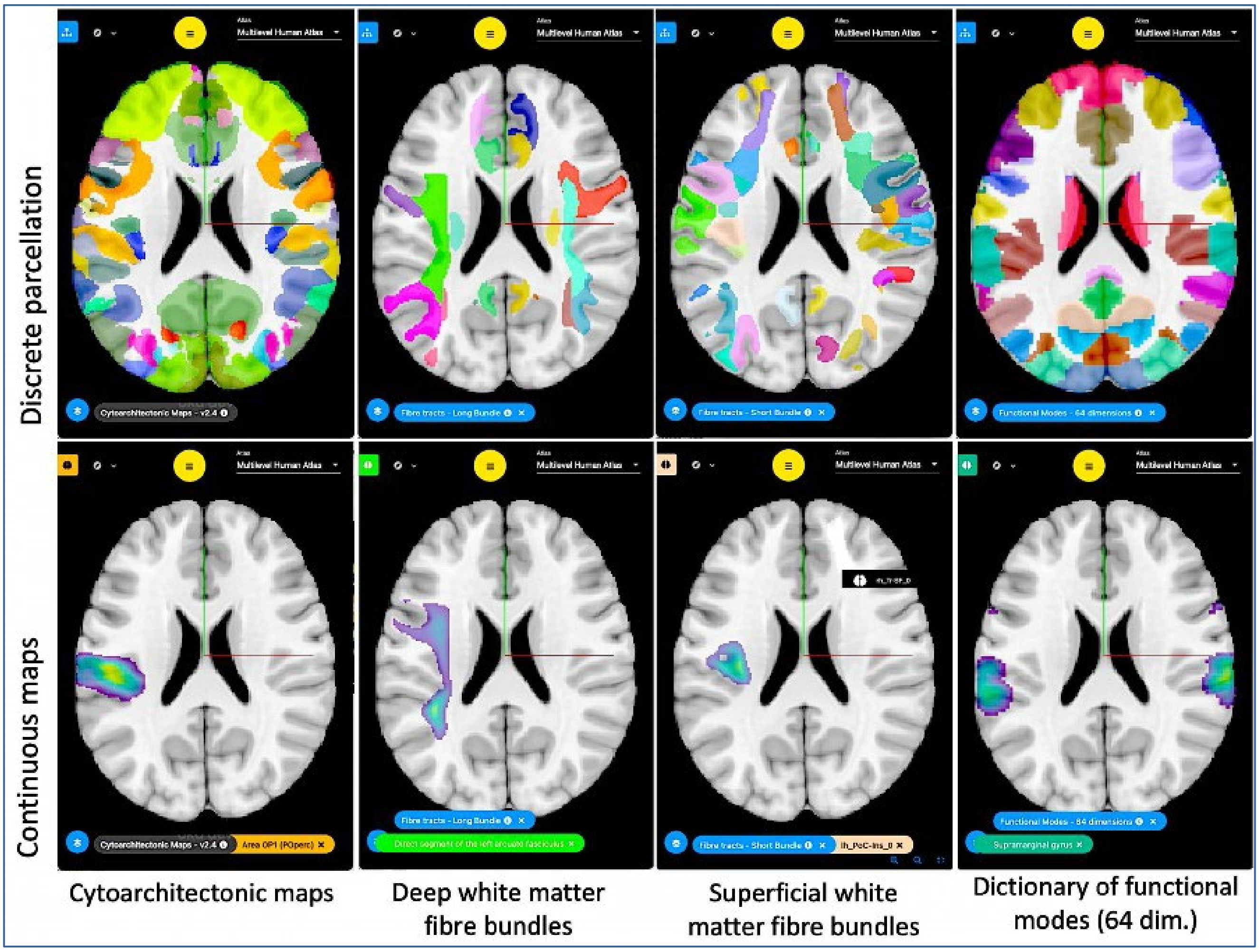
Figure 4: Representing and linking the macroscopic and microscopic scale in the EBRAINS human brain atlas

Top: The MNI spaces and BigBrain model are spatially linked via diffeomorphic transformations with underlying sulcal constraints. Bottom: Cytoarchitectonic maps provide a segregation scheme that is well defined at the cellular level, and at the same time serve as a reference for probabilistic assignment of neuroimaging signals. This way, microstructural definitions of cytoarchitectonic borders serve as a unifying principle across the spatial scales.

However, building a link that bridges multiple spatial scales of the human brain needs to go beyond coordinate transformations, and requires a segregation scheme which is both well-defined at the cellular level and able to localise information at the macroscopic level. This is a key feature of the EBRAINS human brain atlas: it uses the Julich-Brain cytoarchitectonic maps as the unifying principle of organisation, which are precisely defined at the microscopic scale and well established as a reference for anatomical assignment of neuroimaging signals. They are included in the form of probabilistic maps in the MNI spaces - capturing variability across different post-mortem samples -, as well as in the form of a growing set of highly detailed 3D maps that are defined natively for the BigBrain space (Figure 4). Corresponding regions in different templates spaces are linked, so that users can navigate in the same region at different scales (see 2.2.1). The number of cytoarchitectonic region definitions is constantly increasing. The most recent version of the probabilistic maps (Amunts K et al., 2020) (P2583) provides a full coverage of the cortex (see 3.1).

The human brain atlas combines the cytoarchitectonic reference delineations with additional maps describing complementary levels of organisation. These currently include probabilistic maps of deep and superficial white matter fibre bundles, capturing variability and structure of fibre architecture at the macroscopic scale (Guevara M et al., 2017) (P1149). The bundle maps are based on diffusion

MRI and tractography, initially inferred from MRI scans of the ARCHI dataset, and more recently from the Human Connectome Project resulting in more than 700 short bundles. Furthermore, the functional organisation is covered by maps reflecting from dictionaries of functional modes (DiFuMo; (Dadi K et al., 2020)) (P2622) which are offered at different granularities ranging from 64 to 1024 networks. The functional modes are sampled on millions of functional brain (fMRI) volumes, spanned over 27 studies and many research groups. These maps help to solve classic analysis problems on a wide variety of functional images: stimuli decoding, standard GLM analysis of fMRI across sessions and individuals, resting-state functional connectomes biomarkers.



Cytoarchitectonic maps

Deep white matter fibre bundles

Superficial white matter fibre bundles

Dictionary of functional modes (64 dim.)

Figure 5: Complementary maps supported by the multilevel human brain atlas.

Each of the maps is available as a discrete parcellation (top row), or as individual continuous or probabilistic maps (bottom row). The maps are available in the Interactive Atlas Viewer:

https://atlases.ebrains.eu/viewer/saneUrl/icbm_cytomap, https://atlases.ebrains.eu/viewer/saneUrl/icbm_long_bundle, https://atlases.ebrains.eu/viewer/saneUrl/icbm_short_bundle, https://atlases.ebrains.eu/viewer/saneUrl/functional_modes_64

2.1.2 Waxholm Space Rat Atlas

The Waxholm Space (WHS) rat brain reference atlas is defined in a high-resolution volumetric magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) template of an adult (80 days old) Sprague Dawley rat brain (Papp EA et al., 2014). It contains comprehensive anatomical delineations of brain regions and fibre tracts, defined by domain experts interpreting the MRI / DTI template, aided by spatially registered histological images showing the microscopic brain architecture. The atlas is embedded in spatially focused workflows and several other EBRAINS tools (QuickNII, VisuAlign, LocaliZoom, QUINT) for analysing data from the rat brain. Commonly used rat brain reference atlases, including “The rat brain in stereotaxic coordinates” by Paxinos and Watson (editions 2-7) and Swanson’s brain maps: structure of the rat brain (editions 1-4), have been spatially registered to the Waxholm space rat brain atlas, shared via EBRAINS, and incorporated in the EBRAINS data curation workflow. In this way anatomical terms and locations defined using different atlases can be compared and translated across atlases. 2D and 3D image data from 56 datasets, shared via EBRAINS, have been spatially registered to the Waxholm space rat brain atlas.

The first version of the WHS rat brain atlas contained 76 delineations, covering most major brain regions (Papp EA et al., 2014). Version 2 contained 89 delineations and features detailed delineations of the hippocampus and parahippocampal region (Kjonigsen LJ et al., 2015). Version 3 contains 118 structures and includes detailed delineations of the ascending auditory system (Osen KK et al., 2019) (P1879; Figure 6).

When opening the WHS rat brain atlas in the EBRAINS Interactive Atlas Viewer, version 3 is loaded by default (WHS rat brain atlas v3[2]). Earlier versions can be selected and compared. More information about the different versions is available in the EBRAINS Knowledge Graph.

v1.01	v2	v3
https://kg.ebrains.eu/search/instances/Dataset/f40e466b-8247-463a-a4cb-56dfe68e7059	https://kg.ebrains.eu/search/instances/Dataset/2c8ec4fb-45ca-4fe7-accf-c41b5e92c43d	https://kg.ebrains.eu/search/instances/Dataset/e80f9946-1aa9-494b-b81a-9048ca9afdbe
76 region delineations	89 region delineations	118 region delineations

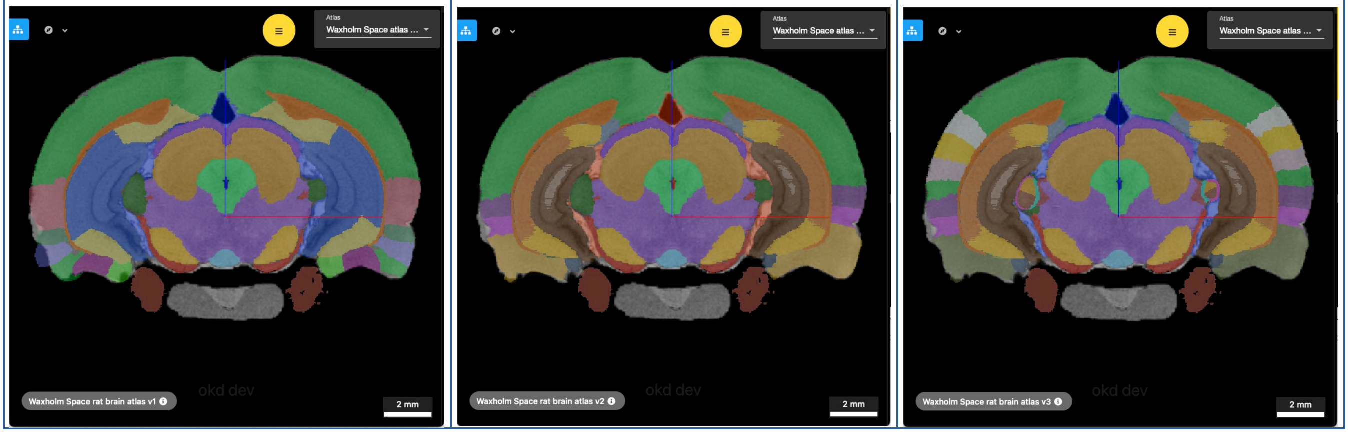


Figure 6: Different versions of the Waxholm Space Atlas of the Sprague Dawley rat brain.

Different version of the Waxholm space atlas of the Sprague Dawley rat brain available in EBRAINS, shown in coronal view at level of the hippocampus in the EBRAINS 3D atlas viewer. The different versions are available in the Interactive Atlas Viewer:

https://atlases.ebrains.eu/viewer/saneUrl/whs_v101,
https://atlases.ebrains.eu/viewer/saneUrl/whs_v2,
https://atlases.ebrains.eu/viewer/saneUrl/whs_v3.

2.1.3 Allen Mouse Brain Atlas

The Allen Mouse Brain Coordinate Framework (CCFv3) has been included in the EBRAINS Atlas services since it is the most widely used open atlas for the mouse brain (Wang Q et al., 2020). The atlas is defined in a population-averaged image volume acquired from 1,675 male C57BL/6J mice using serial two-photon tomography (STPT). The most recent version of the CCFv3 (CCFv3-2017) contains more than 600 brain regions delineated in 3D based on this template. The atlas is used in EBRAINS to compare, analyse, and showcase mouse brain data, and the atlas is embedded in several EBRAINS tools (QuickNII, QUINT workflow). Commonly used mouse brain reference atlases, including “The mouse brain in stereotaxic coordinates” by Paxinos and Watson (editions 2-4), have been spatially registered to the CCFv3, shared via EBRAINS, and incorporated in the EBRAINS data curation workflow. In this way anatomical terms and locations defined using earlier versions of the Franklin & Paxinos atlases can be compared and translated across atlases. A large number of 2D and 3D image

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[2] https://atlases.ebrains.eu/viewer/saneUrl/whs_v3

data shared via EBRAINS have been spatially registered to the CCFv3. When opening the Allen Mouse Brain Coordinate Framework (CCFv3), the user enters the most recent version by default (explore the CCFv3-2017[3]). Earlier versions can be selected and compared. Further details about the CCFv3 can be found on the Allen Brain Atlas website http://atlas.brain-map.org/ of the Allen Institute for Brain Science (Seattle (WA)).

2.2 Exploring atlases

2.2.1 The 3D Interactive Atlas Viewer

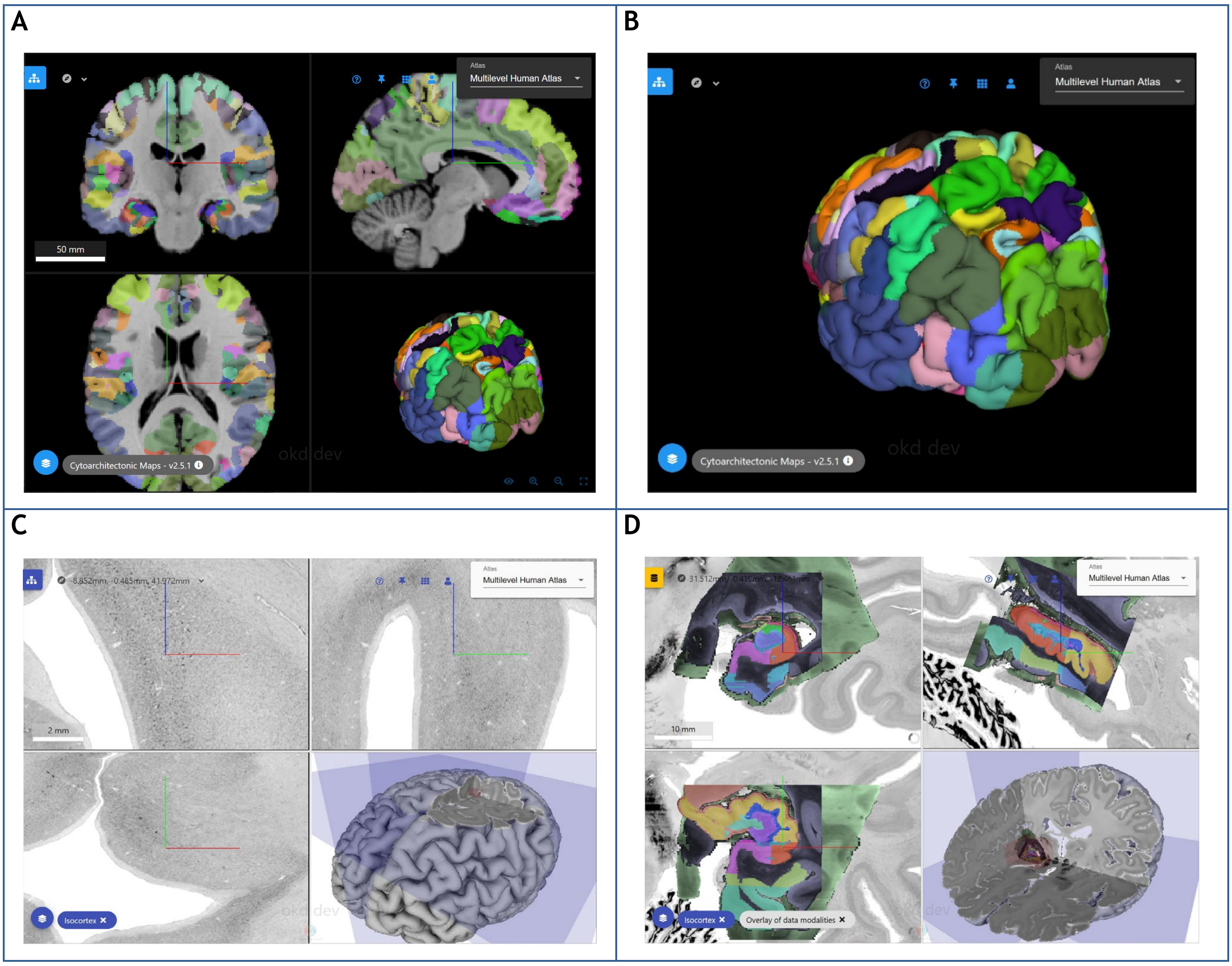

Figure 7: The Interactive Atlas Viewer combines a 3D surface view with three planar views.

(A) MNI reference space of the human atlas with data at about 1mm resolution, displaying the cytoarchitectonic maps. (B) Maximised mode of one view, in this case the 3D rotatable view. In this setting, the “Space” key cycles through the views (https://atlases.ebrains.eu/viewer/saneUrl/colin_cytomap). (C) BigBrain reference space of the human atlas, where the planar views are zoomed into a custom oblique cut at ~20 micrometre resolution, allowing to identify individual cortical layers and larger cells. The 3D view provides orientation about the selected location in the brain (https://atlases.ebrains.eu/viewer/saneUrl/bigbrain_isocortex). (D) Overlay of additional image data, here: High-resolution maps and volumes of interest from MRI and Polarized Light Imaging (https://atlases.ebrains.eu/viewer/saneUrl/bigbrain_3dpli).

The EBRAINS 3D Interactive Atlas Viewer provides an intuitive way of navigating volumetric reference atlases from a web browser. It allows to switch between different template spaces, browse different parcellation maps as visual layers, search their region hierarchies, and select regions to find more

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[3] https://atlases.ebrains.eu/viewer/saneUrl/CCFv3_2017

detailed information and associated data features. The viewer can be reached from the atlas section of the EBRAINS webportal[4], and directly via the URL https://atlases.ebrains.eu/viewer.

At its core, it is a web-based 3D image viewer with a common 2x2 layout that combines a rotatable 3D surface view of a brain volume with three planar views of different, typically orthogonal, image planes (Figure 7A). Each of these four image views allows to zoom (scroll wheel) and pan (click&drag) and can be maximised to full screen (Figure 7B) using the corresponding icon (“⛶”) at the bottom right of each view. The three planar views further allow to select custom oblique sections (shift+click&drag) to produce non-orthogonal views of the 3D image (Figure 7C). This is an important feature especially for high-resolution reference volumes, as it allows to display e.g., cortical layers in their natural orientation - orthogonal to the brain surface - instead of being restricted to axis-aligned coordinate planes.

Building on the open source project Neuroglancer[5], the viewer can visualise very large brain volumes in the Terabyte range, and allows to zoom from common MRI resolutions down to the microscopic resolution of the BigBrain reference space (Figure 7C) without downloading the underlying dataset - which in case of BigBrain typically exceeds the disk capacity of an office computer. The viewer also supports multiple image layers to co-display brain region maps with additional image data (Figure 7D).

The atlas viewer provides various interactive controls for interacting with atlases and their features and gives access to an extensible set of tools for working with data associated to atlas regions, including a “shopping cart” for collecting and downloading interesting data features. Several functionalities have been newly introduced or optimised in the most recent release of the atlas viewer, which is described in more detail in Section 3.2.

Full documentation of the Interactive Atlas Viewer can be found here: https://interactive-viewer.readthedocs.io/en/latest/.

2.2.2 LocaliZoom 2D serial section image viewer

EBRAINS LocaliZoom serial section image viewer provides an intuitive way of navigating high-resolution 2D image series coupled with segmentation overlay, from a web browser. At its core, it is a web-based pan-and-zoom 2D image viewer coupled with a volumetric atlas slicer, and a navigational aid showing the entire image series as a "filmstrip". Building on the open standard Deep Zoom Image format, it is able to efficiently visualise very large brain images in the gigapixel range, allowing to zoom from common, display-sized overview resolutions down to the microscopic resolution without downloading the underlying very large image dataset. It further allows extraction of a limited number of coordinates, e.g. representing an electrode track or labelling within a small region of interest. EBRAINS uses LocaliZoom for inspecting 2D image datasets that are spatially registered to the Waxholm Space rat brain atlas or the Allen Mouse Atlas. Users can inspect the images at cellular resolution and observe brain regions, names, and boundaries and annotate points in order to extract coordinates.

In LocaliZoom, serial section images are represented in a filmstrip at the bottom of the webpage user interface (Figure 8-1), with the selected section image shown with an atlas overlay in the middle panel and the pointer of the mouse showing the atlas region name. In the upper panel, the user can visualise the coordinates of the mouse pointer (Figure 8-2); adjust the transparency of the atlas overlay (Figure 8-3); change the colour of the coordinate markers (Figure 8-4) and export coordinates to either Excel (Figure 8-5) for storage or MeshView for visualisation (Figure 8-6).

The main image can be zoomed and panned by scrolling and clicking. To extract a coordinate, the mouse marker must be positioned at the desired location, and space must be pressed. A cross will appear in the selected colour (Figure 8-4), representing the location of the extracted coordinate. After all desired points have been marked, the coordinates can be exported either to Excel (Figure 8-5) or to MeshView (Figure 8-6) using the corresponding buttons in the upper toolbar. When clicking

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[4] https://ebrains.eu/services/atlases

[5] https://github.com/google/neuroglancer

these buttons, a set of coordinates will appear that can be copy pasted into MeshView in order to work with these point clouds in 3D.

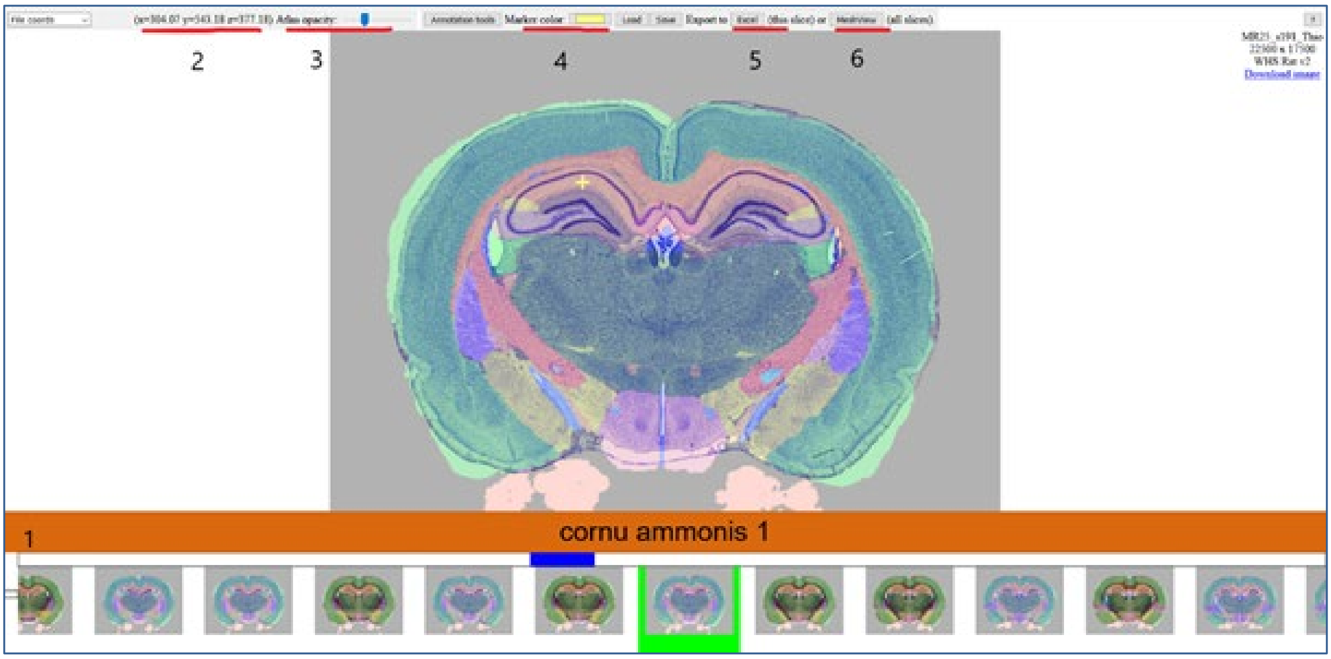
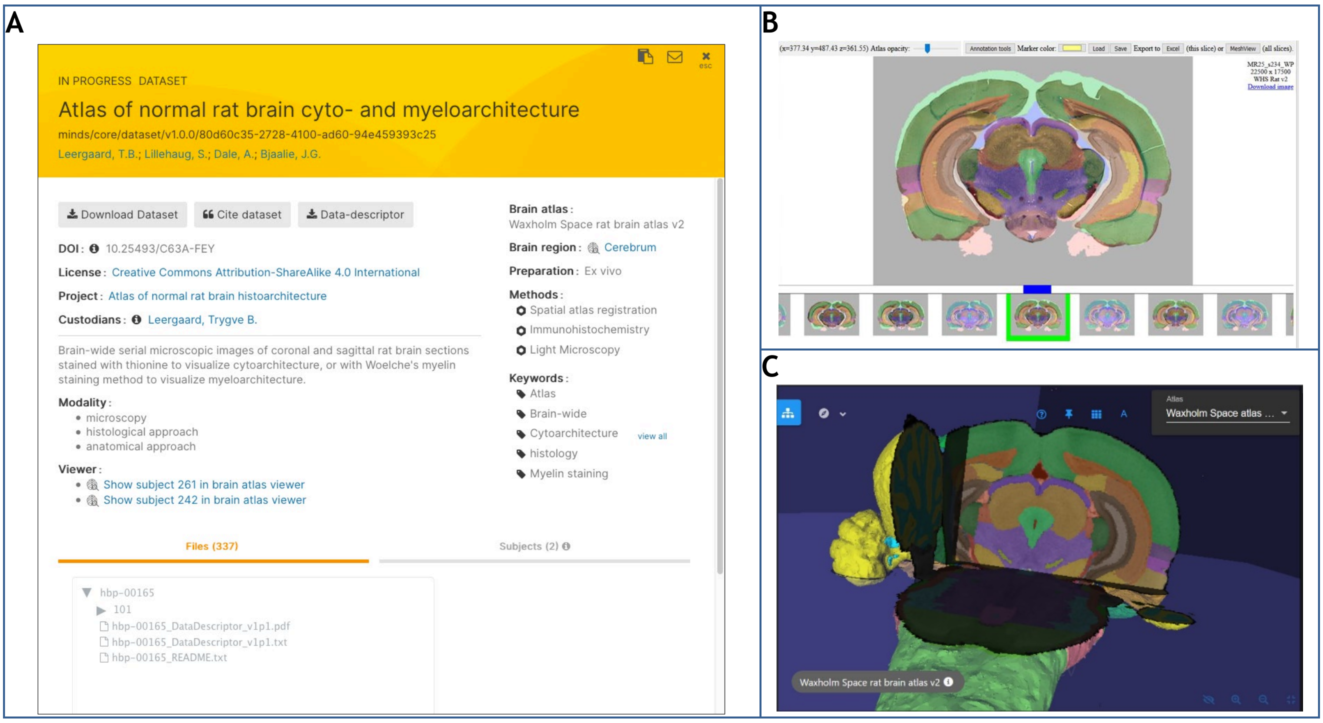


Figure 8: Interface of the LocaliZoom 2D serial section viewer.

Shown here: Waxholm Space rat atlas v2: http://cmbn-navigator.uio.no/navigator/filmstripzoom/filmstripzoom.html?atlas=200000&series=283&preview=WHSRatv2Preview.png.

An example dataset that can be inspected with LocaliZoom is shown in Figure 9 (A,B). Further datasets can be found in the Knowledge Graph Search User Interface using a faceted search function by selecting the filter options “Rattus norvegicus” or “Mus musculus”, and the method “Spatial atlas registration”. More details about the faceted search can be found in “HBP Deliverable 4.1 (D32) - EBRAINS Data and Knowledge services”.



IN PROGRESS DATASET

Atlas of normal rat brain cyto- and myeloarchitecture

minds/core/dataset/v1.0.0/80d60c35-2728-4100-ad60-94e459393c25

Leergaard, T.B.; Lillehaug, S.; Dale, A.; Bjaalie, J.G.

Download Dataset | Cite dataset | Data-descriptor

DOI: 10.25493/C63A-FEY

License: Creative Commons Attribution-ShareAlike 4.0 International

Project: Atlas of normal rat brain histoarchitecture

Custodians: Leergaard, Trygve B.

Brain-wide serial microscopic images of coronal and sagittal rat brain sections stained with thionine to visualize cytoarchitecture, or with Woelche's myelin staining method to visualize myeloarchitecture.

Modality:
- microscopy
- histological approach
- anatomical approach

Viewer:
- Show subject 261 in brain atlas viewer
- Show subject 242 in brain atlas viewer

Brain atlas: Waxholm Space rat brain atlas v2

Brain region: Cerebrum

Preparation: Ex vivo

Methods:
- Spatial atlas registration
- Immunohistochemistry
- Light Microscopy

Keywords:
- Atlas
- Brain-wide
- Cytoarchitecture
- histology
- Myelin staining

view all

Files (337) | Subjects (2)

- hbp-00165
  - 101
  - hbp-00165_DataDescriptor_v1p1.pdf
  - hbp-00165_DataDescriptor_v1p1.txt
  - hbp-00165_README.txt

Figure 9: Viewer links available via the EBRAINS Knowledge Graph.

Where applicable, the EBRAINS Knowledge Graph provides links from Dataset cards (A) to the LocaliZoom 2D serial section image viewer (B) or The 3D Interactive Atlas Viewer (C). The Dataset card with access to the viewers is available here: https://kg.ebrains.eu/search/instances/Dataset/6ce1f96ae210b2335b75a793367e3865.

2.2.3 Access via the EBRAINS Knowledge Graph

DATASET

Waxholm Space atlas of the Sprague Dawley rat brain delineations v3

Osen, K. K.; Imad, J.; Wennberg, A. E.; Papp, E.A.; Leergaard, T.B.

DATASET

Maximum probability map of deep white matter fibre bundles (atlas of deep white matter fibre bundles, version 2018)

Guevara, P.; Duclap, D.; Poupon, C.; Marrakchi-Kacem, L.; Fillard, P.; Le Bihan, D.; Leboyer, M.; Mangin, J. F.

DATASET

Whole-brain parcellation of the Julich-Brain Cytoarchitectonic Atlas (v2.4)

Amunts, K.; Mohlberg, H.; Bludau, S.; Caspers, S.; Eickhoff, S. B.; Pieperhoff, P.

DOI: 10.25493/A7Y0-NX9

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Project: Julich-Brain: cytoarchitectonic probabilistic maps of the human brain

Custodians: Amunts, Katrin

This dataset contains the whole-brain parcellation of the Julich-Brain Cytoarchitectonic Atlas in the MNI Colin 27 as well as the MNI ICBM 152 2009c nonlinear asymmetric reference space (Evans et al., 2012). The parcellation is derived from the individual probability maps (PMs) of the cytoarchitectonic regions released in the Julich-Brain Cytoarchitectonic Atlas, that are further combined into a Maximum Probability Map (MPM). The MPM is calculated by considering for each voxel the probability of all cytoarchitectonic areas released in the atlas, and determining the most probable assignment (Eickhoff 2005). Gap maps complement cortical maps to achieve full cortical coverage. The table "Julich-Brain_shrinkage_corrected_individual_volumes" contains shrinkage corrected individual volumes and hemispheric and gender specific coefficients of variations of all Julich-Brain areas. Note that methodological improvements and integration of new brain structures may lead to small deviations in earlier released datasets.

Modality:
- microscopy
- anatomical approach
- histological approach
- neuroimaging

Brain atlas: Julich-Brain Atlas

Brain region:
- Area 1 (PostCG)
- Area 2 (PostCS)
- Area 25 (sACC)
- Area 33 (ACC)
- Area 3a (PostCG)

Preparation: Ex vivo

Methods:
- cytoarchitectonic mapping
- probability mapping
- maximum probability mapping
- Magnetic Resonance Imaging (MRI)
- silver staining

Keywords:
- brain mapping
- histology
- imaging
- visual system

DATASET

Ultrahigh resolution 3D cytoarchitectonic map of the LGB (lam 1-6, CGL, Metathalamus) created by a Deep-Learning assisted workflow

Schiffer, C.; Brandstetter, A.; Bolakhrif, N.; Mohlberg, H.; Amunts, K.; Dickscheid, T.

DATASET

7T-fMRI data to study mental imagery and illusory perception in the layers of human primary visual cortex (V1)

Bergmann, J.; Morgan, A. T.; Li, M. S.; Muckli, L.

DATASET

Density measurements of different receptors for DG (Hippocampus) [human, v2.0]

Palomero-Gallagher, N.; Zilles, K.

DOI: 10.25493/M8PK-C82

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Project: Quantitative receptor data

Custodians: Zilles, Karl; Palomero-Gallagher, Nicola

This dataset contains the densities (in fmol/mg protein) of receptors for classical neurotransmitters of DG (Hippocampus) obtained by means of quantitative *in vitro* autoradiography. The receptor densities are visualized as *fingerprints* (**fp**), which provide the mean density and standard deviation for each of the analyzed receptor types, averaged across samples.

Overview of available measurements [**receptor** | ***neurotransmitter*** | *labeling agent*]:
AMPA | ***glutamate*** | *[^{3}H]AMPA*
kainate | ***glutamate*** | *[^{3}H]kainate*
NMDA | ***glutamate*** | *[^{3}H]MK-801*
GABA$_A$ | ***GABA*** | *[^{3}H]muscimol*
GABA$_B$ | ***GABA*** | *[^{3}H]CGP54626*
GABA$_A$/BZ | ***GABA*** | *[^{3}H]flumazenil*
muscarinic M$_1$ | ***acetylcholine*** | *[^{3}H]pirenzepine*
muscarinic M$_2$ | ***acetylcholine*** | *[^{3}H]oxotremorine-M*
muscarinic M$_3$ | ***acetylcholine*** | *[^{3}H]4-DAMP*
nicotinic $\alpha_4\beta_2$ | ***acetylcholine*** | *[^{3}H]epibatidine*
α_1 | ***noradrenalin/norepinephrine*** | *[^{3}H]prazosin*
α_2 | ***noradrenalin/norepinephrine*** | *[^{3}H]UK-14,304*
5-HT$_{1A}$ | ***serotonin*** | *[^{3}H]8-OH-DPAT*
5-HT$_2$ | ***serotonin*** | *[^{3}H]ketanserin*
D$_1$ | ***dopamine*** | *[^{3}H]SCH23390*

Brain atlas: Julich-Brain Atlas

Brain region: DG (Hippocampus)

Preparation: Ex vivo

Methods:
- autoradiography - imaging
- autoradiography - quantitative analysis
- Semantic atlas registration

Keywords:
- brain mapping
- histology

Figure 10: Atlas information openly available in the EBRAINS Knowledge Graph.

Almost all information and datasets underlying the EBRAINS atlases are curated and openly accessible as Dataset cards in the EBRAINS Knowledge Graph. Left: Examples of Dataset cards for different parcellations used in EBRAINS atlases. Right: Examples of Dataset cards for multimodal features that are linked to atlas regions. The Dataset cards are available via the Knowledge Graph here: Waxholm Space atlas of the Sprague Dawley rat brain delineations v3 - https://kg.ebrains.eu/search/instances/Dataset/e80f9946-1aa9-494b-b81a-9048ca9afdbe, Maximum probability map of deep white matter fibre bundles (atlas of deep white matter fibre bundles, version 2018) - https://kg.ebrains.eu/search/instances/Dataset/fcbb049b-edd5-4fb5-acbc-7bf8ee933e24, Whole-brain parcellation of the Julich-Brain Cytoarchitectonic Atlas (v2.4) - https://kg.ebrains.eu/search/instances/Dataset/5249afa7-5e04-4ffd-8039-c3a9231f717c, Ultrahigh resolution 3D cytoarchitectonic map of the LGB (lam 1-6, CGL, Metathalamus) created by a Deep-Learning assisted workflow - https://kg.ebrains.eu/search/instances/Dataset/d0c36f4a-91a8-4885-880d-f2896f5c54cf, 7T-fMRI data to study mental imagery and illusory perception in the layers of human primary visual cortex (V1) - https://kg.ebrains.eu/search/instances/Dataset/de7a6c44-8167-44a8-9cf4-435a3dab61ed, Density measurements of different receptors for DG (Hippocampus) [human, v2.0] - https://kg.ebrains.eu/search/instances/Dataset/e1ae30a5-f6ed-40f8-ad54-9ded33b82052.

EBRAINS brain atlases can also be accessed via the EBRAINS Knowledge Graph (KG) Search User Interface at https://kg.ebrains.eu/search, because almost all of the parcellations, maps, reference templates and multimodal datasets that make up the atlases are curated and indexed in the KG in accordance with the FAIR principles (cf. D4.1 EBRAINS Data and Knowledge services). While the KG Search User Interface is not designed as an atlas exploration tool, it allows several workflows that make use of atlas information and related metadata:

- In the KG, the new schemas of the openMINDS SANDS metadata model allow to capture and query information on the anatomical location of neuroscience data as well as to identify datasets that are part of reference brain atlases and coordinate spaces. The previous version of the KG metadata models allowing tagging parcellations, brain regions, and coordinate spaces.
- Some dataset information pages in the KG provide direct URL links to view the corresponding data in an appropriate online viewer (as in Figure 10)

- Complex brain atlases that sometimes are comprised of multiple dataset releases can be captured as individual datasets within the KG, allowing to model the provenance of the brain atlas and explore the history and related publications (see 3.1)

2.2.4 Python Client and HTTP API

EBRAINS develops a dedicated HTTP API and Python Client for working with atlases in a more programmatic fashion. The Python client is currently at the prototype stage and has been used for a first coupling of brain simulation with the EBRAINS atlases (Showcase 1 of WP 1). A brief outline of this new development is included in Section 3.3.

2.3 Integration of multimodal data to atlases

2.3.1 Datasets as multimodal features of brain regions

Together with the curation teams of Service Category 1 (SC1), brain atlases are continuously enriched with multimodal information by linking neuroscience datasets to their most appropriate locations in an atlas (Tier 2 curation). The assignment of location can take different forms:

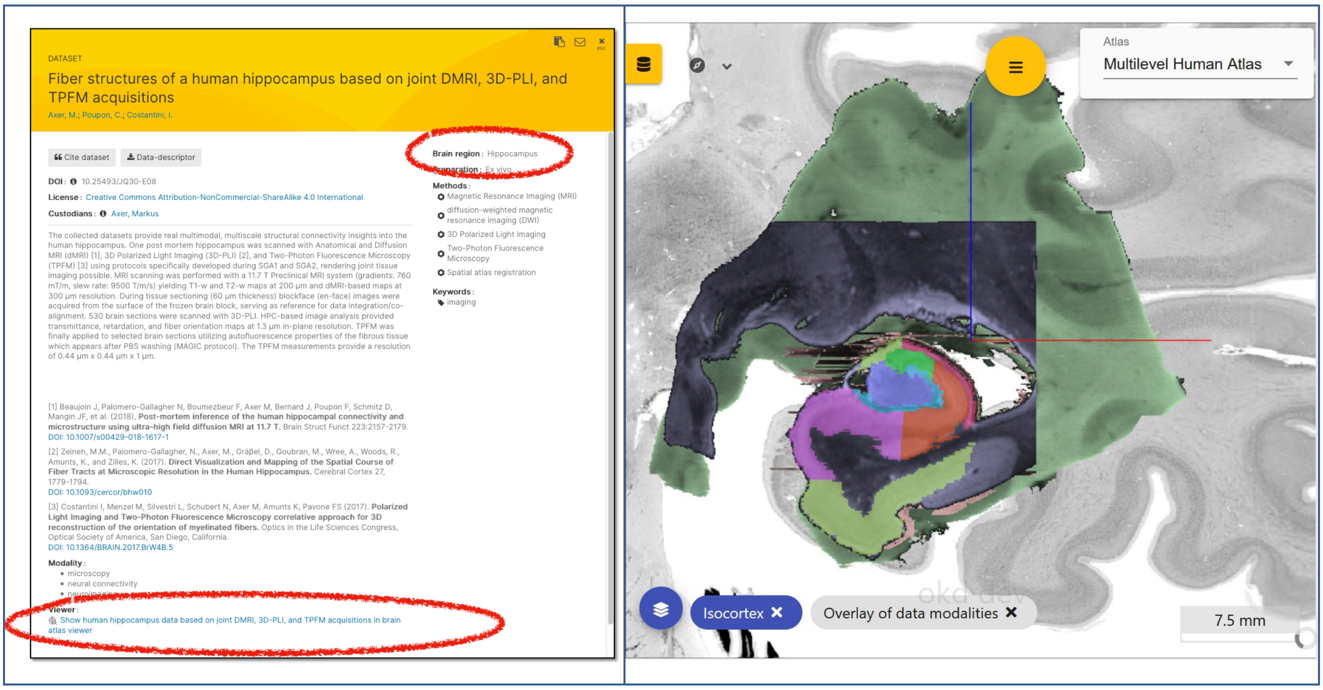
1) Spatial registration of coordinates to one of the supported reference spaces (“coordinate-based integration”). This applies to 2D and 3D images, point locations like recording sites of tissue probes, or geometric shapes like neuron morphologies (see 2.3).
2) Direct assignment to a brain region (“semantic integration”). In this case, the origin brain region of a dataset is known, and the dataset is linked to the name of the region according to a reference parcellation.
3) Probabilistic assignment of whole brain signals to brain regions. This applies to continuous feature distributions in the form of images in a reference space, as for example 3D activation maps from fMRI studies. Here the coverage of certain brain regions by the signal can be evaluated from a probabilistic perspective, leading to multi-assignments with probabilistic weights to different brain regions. This corresponds to the concept used in the SPM anatomy toolbox (Eickhoff S et al., 2005). The human atlas currently provides links of this type to a range of fMRI scans stored in the EBRAINS Knowledge Graph.

More details regarding the data curation processes can be found in “HBP Deliverable 4.1 (D32) - EBRAINS Data and Knowledge services”. EBRAINS makes links between datasets and brain locations accessible in different ways:

- As a region specification appearing in the corresponding dataset description of the EBRAINS Knowledge Graph (Figure 11 left)
- As a direct link from the dataset description in the EBRAINS Knowledge Graph to a superimposed view in EBRAINS atlas viewer (Figure 11 right)
- As a list of data features presented in the 3D atlas viewer when selecting a brain region (Figure 12)

In addition, a Python client and HTTP API are in development, that will allow to retrieve datasets by brain region in a programmatic fashion (see 2.2.4).

The number of datasets linked to brain regions is constantly increasing and kept up to date with modifications of the atlas parcellations and maps. For example, browsing regions of the Julich-Brain cytoarchitectonic maps includes links to 214 datasets with regional maps, 24 datasets from molecular imaging, 4 datasets with single cell measurements, 4 physiology datasets, 3 high-resolution MRI datasets, several microscopy datasets and imaging studies, whole-brain connectivity from 5 different cohort studies, and retrieval of gene expressions from the Allen brain atlas for each individual brain region. In this way, it provides access to about 20,000 files with locations.



DATASET

Fiber structures of a human hippocampus based on joint DMRI, 3D-PLI, and TPFM acquisitions

Axer, M.; Poupon, C.; Costantini, I.

Cite dataset | Data-descriptor

DOI: 10.25493/JQ30-E08

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Custodians: Axer, Markus

The collected datasets provide real multimodal, multiscale structural connectivity insights into the human hippocampus. One post mortem hippocampus was scanned with Anatomical and Diffusion MRI (dMRI) [1], 3D Polarized Light Imaging (3D-PLI) [2], and Two-Photon Fluorescence Microscopy (TPFM) [3] using protocols specifically developed during SGA1 and SGA2, rendering joint tissue imaging possible. MRI scanning was performed with a 11.7 T Preclinical MRI system (gradients: 760 mT/m, slew rate: 9500 T/m/s) yielding T1-w and T2-w maps at 200 µm and dMRI-based maps at 300 µm resolution. During tissue sectioning (60 µm thickness) blockface (en-face) images were acquired from the surface of the frozen brain block, serving as reference for data integration/co-alignment. 530 brain sections were scanned with 3D-PLI. HPC-based image analysis provided transmittance, retardation, and fiber orientation maps at 1.3 µm in-plane resolution. TPFM was finally applied to selected brain sections utilizing autofluorescence properties of the fibrous tissue which appears after PBS washing (MAGIC protocol). The TPFM measurements provide a resolution of 0.44 µm x 0.44 µm x 1 µm.

[1] Beaujoin J, Palomero-Gallagher N, Boumezbeur F, Axer M, Bernard J, Poupon F, Schmitz D, Mangin JF, et al. (2018), **Post-mortem inference of the human hippocampal connectivity and microstructure using ultra-high field diffusion MRI at 11.7 T.** Brain Struct Funct 223:2157-2179. DOI: 10.1007/s00429-018-1617-1

[2] Zeineh, M.M., Palomero-Gallagher, N., Axer, M., Gräßel, D., Goubran, M., Wree, A., Woods, R., Amunts, K., and Zilles, K. (2017). **Direct Visualization and Mapping of the Spatial Course of Fiber Tracts at Microscopic Resolution in the Human Hippocampus.** Cerebral Cortex 27, 1779-1794. DOI: 10.1093/cercor/bhw010

[3] Costantini I, Menzel M, Silvestri L, Schubert N, Axer M, Amunts K, Pavone FS (2017). **Polarized Light Imaging and Two-Photon Fluorescence Microscopy correlative approach for 3D reconstruction of the orientation of myelinated fibers.** Optics in the Life Sciences Congress, Optical Society of America, San Diego, California. DOI: 10.1364/BRAIN.2017.BrW4B.5

Modality:
- microscopy
- neural connectivity
- neuroimaging

Viewer:
Show human hippocampus data based on joint DMRI, 3D-PLI, and TPFM acquisitions in brain atlas viewer

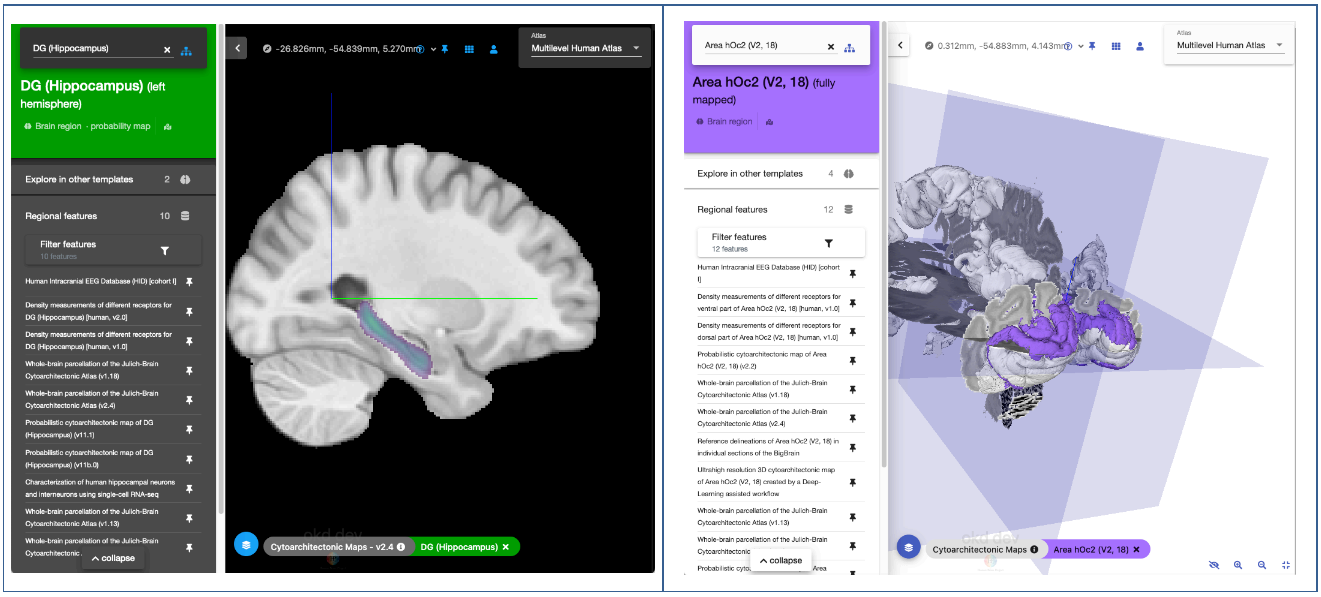
Brain region: Hippocampus
Preparation: Ex vivo

Methods:
- Magnetic Resonance Imaging (MRI)
- diffusion-weighted magnetic resonance imaging (DWI)
- 3D Polarized Light Imaging
- Two-Photon Fluorescence Microscopy
- Spatial atlas registration

Keywords:
- imaging

Figure 11: Accessibility of region specifications for a dataset in the EBRAINS Knowledge Graph.

Left: The associated brain region names are listed on the Dataset card. If applicable, an additional link allows to open a 3D view of files from the dataset in EBRAINS atlas viewer. Right: Dataset view in the EBRAINS Interactive Atlas Viewer. Files of the dataset are displayed at their position in reference space (here: BigBrain reference space). The Dataset card with access to the Interactive Atlas Viewer is available via the EBRAINS Knowledge Graph here: https://kg.ebrains.eu/search/instances/Dataset/b08a7dbc-7c75-4ce7-905b-690b2b1e8957.



Human Intracranial EEG Database (HID) [cohort I]
Density measurements of different receptors for DG (Hippocampus) [human, v2.0]
Density measurements of different receptors for DG (Hippocampus) [human, v1.0]
Whole-brain parcellation of the Julich-Brain Cytoarchitectonic Atlas (v1.18)
Whole-brain parcellation of the Julich-Brain Cytoarchitectonic Atlas (v2.4)
Probabilistic cytoarchitectonic map of DG (Hippocampus) (v11.1)
Probabilistic cytoarchitectonic map of DG (Hippocampus) (v11b.0)
Characterization of human hippocampal neurons and interneurons using single-cell RNA-seq
Whole-brain parcellation of the Julich-Brain Cytoarchitectonic Atlas (v1.13)
Whole-brain parcellation of the Julich-Brain Cytoarchitectonic

Human Intracranial EEG Database (HID) [cohort I]
Density measurements of different receptors for ventral part of Area hOc2 (V2, 18) [human, v1.0]
Density measurements of different receptors for dorsal part of Area hOc2 (V2, 18) [human, v1.0]
Probabilistic cytoarchitectonic map of Area hOc2 (V2, 18) (v2.2)
Whole-brain parcellation of the Julich-Brain Cytoarchitectonic Atlas (v1.18)
Whole-brain parcellation of the Julich-Brain Cytoarchitectonic Atlas (v2.4)
Reference delineations of Area hOc2 (V2, 18) in individual sections of the BigBrain
Ultrahigh resolution 3D cytoarchitectonic map of Area hOc2 (V2, 18) created by a Deep-Learning assisted workflow
Whole-brain parcellation of the Julich-Brain Cytoarchitectonic Atlas (v1.13)
Whole-brain parcellation of the Julich-Brain Cytoarchitectonic
Probabilistic cyto Area

Figure 12: Accessing multimodal datasets linked to brain regions in Interactive Atlas Viewer.

Left: Probability map of area DG in MNI reference space with associated data features (https://atlases.ebrains.eu/viewer/saneUrl/icbm_dg). Right: High-resolution map of area hOc2 (V2, Brodmann Area 18) in BigBrain reference space with associated data features (https://atlases.ebrains.eu/viewer/saneUrl/bigbrain_hoc2).

2.3.2 *Integrating 2D images using QuickNII and VisuAlign*

Tools for anatomical landmark-based registration of mouse and rat 2D brain images to 3D murine reference atlases are QuickNII and VisuAlign (Puchades MA et al., 2019) (P2182). The atlases embedded in these tools are Allen Mouse Brain Atlas (CCFv3, 2015 and 2017) and the Waxholm Atlas of the Spraque Dawley rat (v2 and v3).

QuickNII is a stand-alone software tool for semi-automated affine registration of 2D brain section images to mouse and rat reference atlases. It has been used for integration of more than 100 2D datasets, available in the EBRAINS Knowledge Graph. Based on landmarks visible in the tissue, the user anchors some of the images from a series of sections to reference slices, and the propagation algorithm will position the remaining images of the series. Further precision is achieved with the VisuAlign software which refines the QuickNII registration of the images using non-linear deformation (Figure 13).

Both tools are available for Windows and OS operative systems and shared from the EBRAINS web portal: https://ebrains.eu/service/quicknii-and-visualign

Tutorials and manuals for the tools can be found in a publicly available Collab: https://wiki.ebrains.eu/bin/view/Collabs/quicknii-and-visualign/

QuickNII and VisuAlign are key elements of the QUINT workflow ((Yates SC et al., 2019) (P2245), (Groeneboom NE et al., 2020) (P2589)), allowing users to perform atlas-based quantifications of the labelled features in their brain sections.

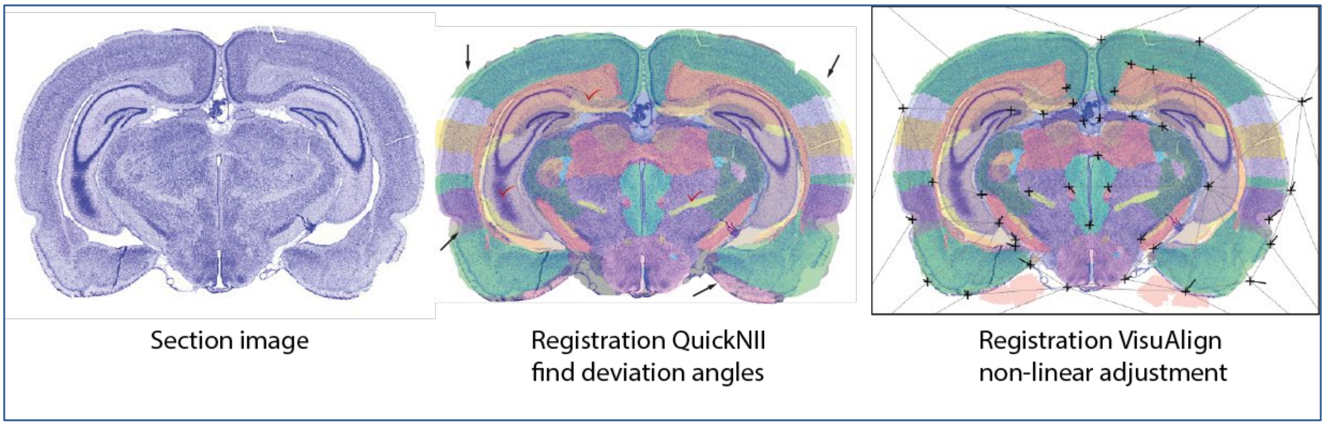


Figure 13: Registration of 2D histological images with QuickNII and VisuAlign.

2.3.3 Integrating high-resolution partial volumes - VoluBA

Spatial anchoring of high-resolution volumes of interest (VOIs) from specific imaging experiments into the detailed anatomical context of a high-resolution reference model like the BigBrain is an important practical problem since such high-resolution models and VOIs become increasingly available in recent years. Typical application examples are integration of laminar resolution functional MRI (De Martino F et al., 2013), microscopic resolution structural connectivity as derived from 3D Polarized Light Imaging (Axer M et al., 2011), or 2-Photon fluorescence imaging (e.g., (Silvestri L et al., 2014)), which would all be appropriately organised in the detailed anatomical context of the BigBrain microscopic brain model. However, downloading this huge (~1 Terabyte) dataset and working on a proper positioning of new imaging data is out of reach for many neuroscientists due to the sheer size of the data, as well as the lack of practical tools.

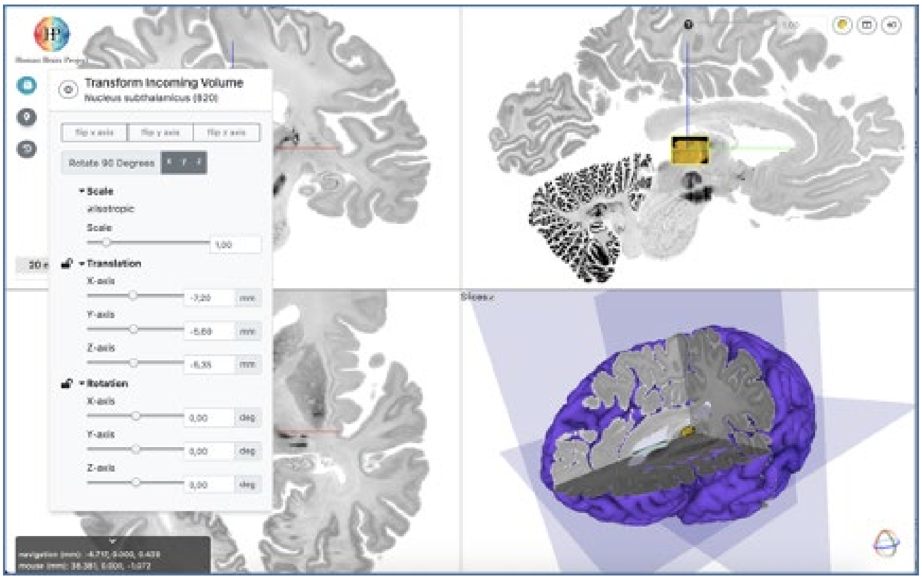
EBRAINS atlas services provide an interactive online service for the anchoring of user data to very large reference volumes, *VoluBA* (Volumetric Brain Anchoring). VoluBA is accessible from https://voluba.apps.hbp.eu. VoluBA is implemented as an online web service that currently provides the BigBrain model as a fixed reference dataset and allows users to upload data from their own imaging experiment to perform interactive anchoring. VoluBA shares several software components and its overall look & feel with the The 3D Interactive Atlas Viewer, so that a user of the EBRAINS atlas services will quickly feel comfortable with it. Furthermore, the image data is read from the same image service, so that a dataset in VoluBA can be directly set as an image source of the atlas viewer and viewed as an image overlay in atlas context.

The anchoring process of a VOI is typically started by direct manipulation of its position and 3D orientation using the mouse pointer. Additional linear transformations like flipping of coordinate axes and isotropic or anisotropic scaling are accessible from a menu (Figure 14). This initial alignment results in a rigid transformation from the VOI to the reference volume. Furthermore, VoluBA supports full 3D affine transformations, allowing more precise adjustment with shearing parameters. This is achieved by interactive anchoring of pairs of corresponding 3D landmarks to the scene (Figure 15).

The resulting linear transformation can be downloaded and/or submitted to EBRAINS data curation. The aligned VOI can be opened in the EBRAINS Interactive Atlas Viewer to see the aligned data in context with brain region maps and other datasets.

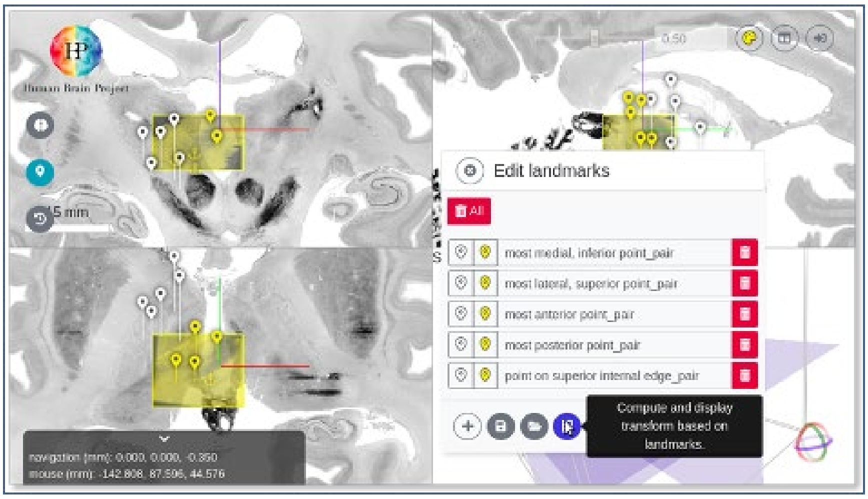
In addition to this, an experimental plugin is available which allows a nonlinear adjustment of cortical VOIs, provided that a segmentation mask for the grey matter is available as well. After an initial linear alignment of the cortical VOI to its target position and orientation, this plugin will then try to adjust the cortical depth of the VOI according to the equivolumetric model of Bok to match that of the reference model. This allows a much better alignment of cortical layers across different subjects.

Full documentation on the Volumetric Brain Anchoring tool can be found here: https://voluba.readthedocs.io/en/latest/.



1,00
-7,20 mm
-5,60 mm
-6,35 mm
0,00 deg
0,00 deg
0,00 deg

Figure 14: Rigid alignment of a high-resolution volume of interest to BigBrain using VoluBA.



most medial, inferior point_pair
most lateral, superior point_pair
most anterior point_pair
most posterior point_pair
point on superior internal edge_pair

navigation (mm): 0.000, 0.000, -0.350
mouse (mm): -142.808, 87.596, 44.576

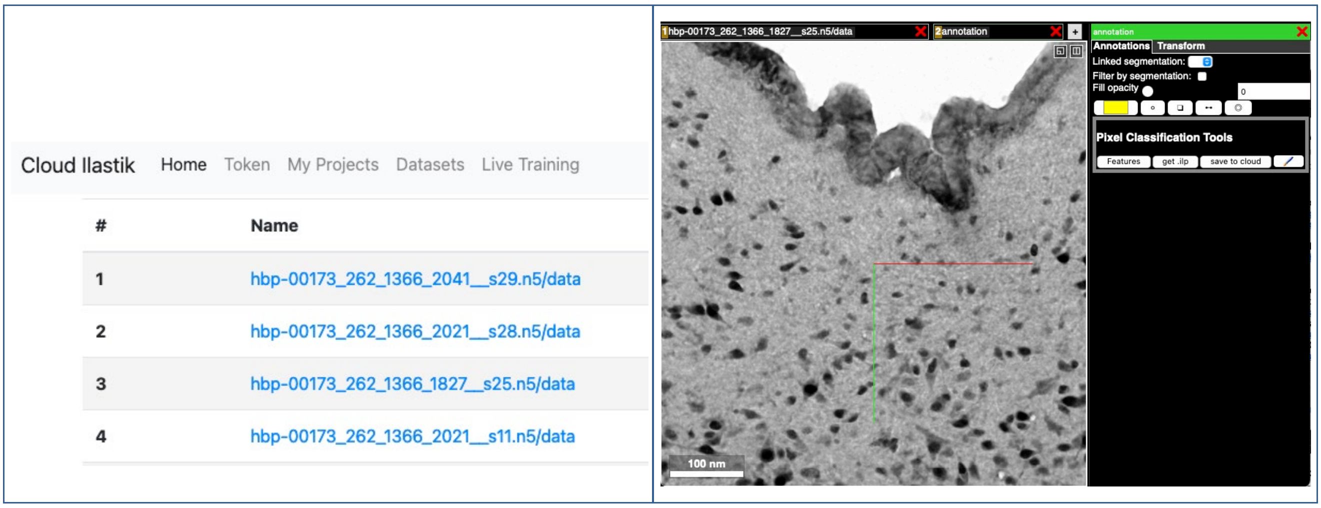
Figure 15: Refining a rigid alignment in VoluBA by entering corresponding 3D landmarks.

2.4 Tools for analysing data using EBRAINS atlases

2.4.1 Interactive image segmentation using ilastik

Ilastik is a simple, user-friendly tool for interactive image classification, segmentation, and analysis. It is built as a modular software framework, which currently has workflows for automated (supervised) pixel- and object-level classification, automated and semi-automated object tracking, semi-automated segmentation, and object counting without detection. Most analysis operations are performed lazily, which enables targeted interactive processing of data subvolumes, followed by complete volume analysis in offline batch mode. Using it requires no experience in image processing. Ilastik version 1.0 is described in a recent publication (Berg S et al., 2019) (P2348). The common form of ilastik is a Desktop application which can be installed on most common operating systems. It is available from https://www.ilastik.org.

EBRAINS builds interfaces with ilastik to allow segmentation of image data from the EBRAINS Knowledge Graph, and to enable execution of image segmentation tasks on EBRAINS compute resources. Two interfaces are currently available in prototype form. The first is a data import module which allows to load image data from EBRAINS storage systems into the ilastik Desktop client, allowing to apply any of the available ilastik workflows to EBRAINS image datasets. Installing and using this prototype is documented in a public EBRAINS collab[6]. The second form - *Cloud ilastik* - is a web version of the ilastik client with reduced functionality, which does not require installation of the client. This prototype runs in a web browser and allows to train a “pixel classification” classifier in a web browser and store the resulting model in EBRAINS (Figure 16). This prototype is accessible at https://web.ilastik.org/live_training/.



#	Name
1	hbp-00173_262_1366_2041__s29.n5/data
2	hbp-00173_262_1366_2021__s28.n5/data
3	hbp-00173_262_1366_1827__s25.n5/data
4	hbp-00173_262_1366_2021__s11.n5/data

Figure 16: The *cloud ilastik* prototype for online interactive image segmentation.

Left: Starting page for the “Live training” allows selection of suitable image datasets. Right: Interactive web interface for performing the training and storing the trained classifier in, based on Neuroglancer[7].

2.4.2 Extracting image features in atlas space - QUINT

QUINT is a workflow for the quantification and spatial analysis of labelled features in a large number of histological brain sections. The workflow consists of several consecutive software tools. All applications are desktop based and have user-friendly graphical interfaces and tutorials. First, QuickNII and VisuAlign (Puchades MA et al., 2019) (P2182) provide precise image registration, ilastik (Berg S et al., 2019) (P2348) allows the segmentation of marked objects in the brain section, and then Nutil (Groeneboom NE et al., 2020) (P2589) subsequently quantifies features per identified

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[6] https://collab.humanbrainproject.eu/#/collab/11516/nav/86128
[7] https://github.com/google/neuroglancer

atlas region (Figure 17). Added value of the QUINT workflow is that it removes the need for time-consuming methods such as stereotactic analysis with manual delineation of the individual brain regions.

- QuickNII and VisuAlign allow precise registrations of 2D brain image data to reference atlases both for mice and rats. Any deviation in cutting angle can be accepted and the propagation algorithm greatly facilitates registrations of brain-wide series with hundreds of slices.
- The output of QuickNII and VisuAlign are tailor-made for input into the quantification workflow QUINT but can also be used directly or in combination with other workflows.
- The QUINT workflow is unique as it allows the users to customise their analysis in many aspects like choice of the granularity level of the atlas, or define their own ROI, filtrate artefacts and quality control steps included in the workflow.

All software tools can be downloaded via the EBRAINS web portal: https://ebrains.eu/service/quint. A manual for the tools can be found in this publicly available collab: https://wiki.ebrains.eu/bin/view/Collabs/quint

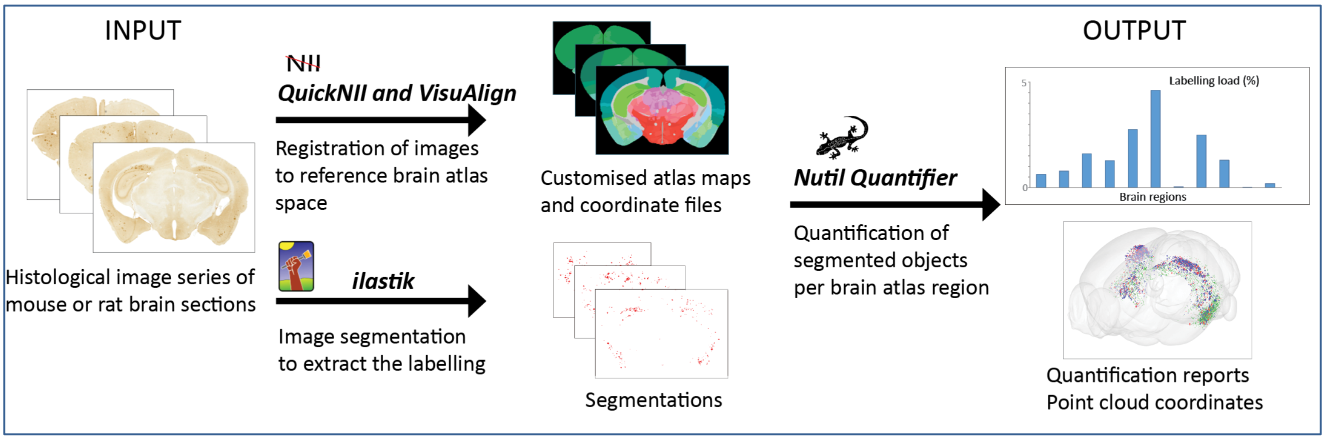

Figure 17: QUINT workflow for spatial feature analysis in rodent brain histological series.

2.4.3 Gene expression analysis in brain regions - JuGEx

Decoding the chain from genes to cognition requires detailed insights how areas with specific gene activities and microanatomical architectures contribute to brain function and dysfunction. The Allen Human Brain Atlas[8] contains regional gene expression data, while the EBRAINS human brain atlas offers three-dimensional cytoarchitectonic maps reflecting the interindividual variability. JuGEx offers an integrated framework that combines the analytical benefits of both repositories towards a multilevel brain atlas of adult humans. It is a new method for integrating tissue transcriptome and cytoarchitectonic segregation.

EBRAINS atlas services provide JuGEx in two forms: a plugin of the Interactive Atlas Viewer (Figure 18 left), as well as a Python library accessible via the Collaboratory (Figure 18 right), thus allowing for both interactive and advanced programmatic use. For the plugin, the python code was wrapped into a back end web service that is interactively controlled via the plugin, providing an intuitive graphical user interface that allows visual selection of brain areas and auto-completion for selecting candidate genes. Once an experiment is configured, it can be executed directly from the viewer. The resulting probe locations can be visualised as landmarks (Figure 18 left), and all results can be downloaded as a .csv file. On request, the Plugin can generate the explicit Python code that corresponds to this computation, and directly import it into a private user Collab for programmatic use in a Jupyter notebook. This allows a seamless transition from basic exploratory analysis to larger scale data experiments. The EBRAINS interfaces to JuGEx are described on the EBRAINS portal at

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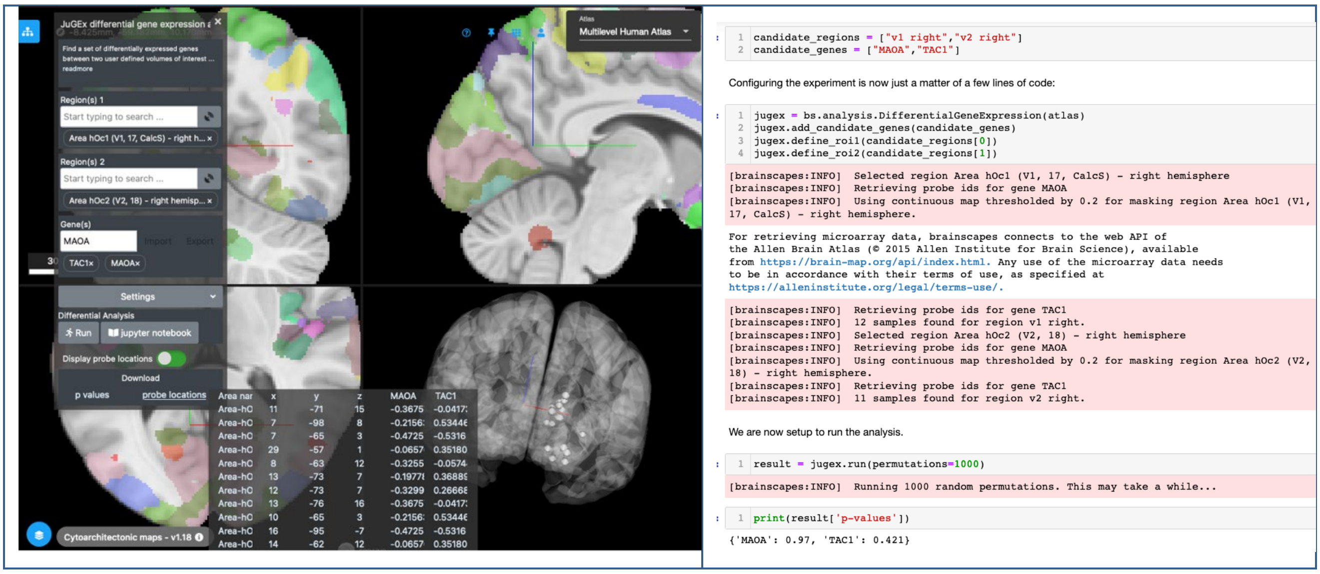
[8] © 2015 Allen Institute for Brain Science. Allen Brain Atlas API. Available from: brain-map.org/api/index.html

https://ebrains.eu/service/jugex, where links to documentation and software are continuously updated.



JuGEx differential gene expression a

Find a set of differentially expressed genes between two user defined volumes of interest ... readmore

Region(s) 1
Area hOc1 (V1, 17, CalcS) - right h...

Region(s) 2
Area hOc2 (V2, 18) - right hemisp...

Gene(s)
MAOA
TAC1
MAOA

Differential Analysis

Display probe locations

Area nar	x	y	z	MAOA	TAC1
Area-hO	11	-71	15	-0.3675	-0.0417
Area-hO	7	-98	8	-0.2156	0.5344
Area-hO	7	-65	3	-0.4725	-0.5316
Area-hO	29	-57	1	-0.0657	0.35180
Area-hO	8	-63	12	-0.3255	-0.0574
Area-hO	13	-73	7	-0.1977	0.3688
Area-hO	12	-73	7	-0.3299	0.2666
Area-hO	13	-76	16	-0.3675	-0.0417
Area-hO	10	-65	3	-0.2156	0.5344
Area-hO	16	-95	-7	-0.4725	-0.5316
Area-hO	14	-62	12	-0.0657	0.35180

```
candidate_regions = ["v1 right","v2 right"]
candidate_genes = ["MAOA","TAC1"]
```

Configuring the experiment is now just a matter of a few lines of code:

```
jugex = bs.analysis.DifferentialGeneExpression(atlas)
jugex.add_candidate_genes(candidate_genes)
jugex.define_roi1(candidate_regions[0])
jugex.define_roi2(candidate_regions[1])
```

```
[brainscapes:INFO]  Selected region Area hOc1 (V1, 17, CalcS) - right hemisphere
[brainscapes:INFO]  Retrieving probe ids for gene MAOA
[brainscapes:INFO]  Using continuous map thresholded by 0.2 for masking region Area hOc1 (V1,
17, CalcS) - right hemisphere.

For retrieving microarray data, brainscapes connects to the web API of
the Allen Brain Atlas (© 2015 Allen Institute for Brain Science), available
from https://brain-map.org/api/index.html. Any use of the microarray data needs
to be in accordance with their terms of use, as specified at
https://alleninstitute.org/legal/terms-use/.

[brainscapes:INFO]  Retrieving probe ids for gene TAC1
[brainscapes:INFO]  12 samples found for region v1 right.
[brainscapes:INFO]  Selected region Area hOc2 (V2, 18) - right hemisphere
[brainscapes:INFO]  Retrieving probe ids for gene MAOA
[brainscapes:INFO]  Using continuous map thresholded by 0.2 for masking region Area hOc2 (V2,
18) - right hemisphere.
[brainscapes:INFO]  Retrieving probe ids for gene TAC1
[brainscapes:INFO]  11 samples found for region v2 right.
```

We are now setup to run the analysis.

```
result = jugex.run(permutations=1000)
```

```
[brainscapes:INFO]  Running 1000 random permutations. This may take a while...
```

```
print(result['p-values'])
```

```
{'MAOA': 0.97, 'TAC1': 0.421}
```

Figure 18: JuGEx, a tool for analysing gene expressions in different atlas regions.

Left: JuGEx integrated as an interactive plugin of the EBRAINS atlas viewer. Right: The same code as a Python notebook in a Jupyter notebook of the EBRAINS Collaboratory.

3. Recent developments

3.1 Updated brain reference atlases

The most notable development for the human atlas since April 2020 is the integration of the new Julich-Brain v2.5 (Amunts K et al., 2020) (P2583), which contains probabilistic cytoarchitectonic maps of 248 different cortical and subcortical areas (Figure 19 left). It brings together brain region definitions from 41 projects of doctoral students and researchers and integrates in total 85,210 contour lines from histological sections into the 3D reference framework. Besides adding many more brain regions, this new release provides whole-brain coverage of the cortical surface. About 70% of the cortical surface are now covered with published mapping projects, and complemented with several "gap maps" which pool the remaining uncharted areas. The gap maps will be continuously refined with ongoing and new mapping projects and released in EBRAINS. The new Julich-Brain is accessible as part of the multilevel atlas in the 3D atlas viewer[9], and as a data project in the EBRAINS Knowledge Graph (see 2.2.3). The new brain areas included in this version include:

- Area TI (STG) (v5.1) 48383281-9a83-4c6f-8166-f8bb139052dd
- Area TE 2.1 (STG) (v5.1) a5589ffe-5148-4006-8d8d-8bcf411f750b
- Area TE 2.2 (STG) (v5.1) d5bfb044-ec4e-4fe6-ad9c-844cad8c7844
- Area TeI (STG) (v5.1) 35f2107d-2e3c-41ae-b26a-83b101829346
- GapMap Frontal II (v9.0) 86510749-dec2-4dff-bc3a-f9fb98c5447d
- GapMap Frontal I (v9.0) 7383f4c6-d678-4539-8d36-33501eef55d3
- GapMap Frontal to Occipital (v9.0) 83b4b184-57be-4385-a1b2-2cec8519509d
- GapMap Frontal to Temporal (v9.0) 48f5905e-2cc5-41ae-b37a-6eb1438337c5

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[9] https://atlases.ebrains.eu/viewer/saneUrl/icbm_cytomap

- GapMap Temporal to Parietal (v9.0) 822ef227-264a-4584-8c3b-e41e33e16fe7
- CA1 (Hippocampus) (v11.1) effec48c-edf6-4be4-9e0e-75c57480d53c
- CA2 (Hippocampus) (v11.1) 159b16ee-2451-44d6-81cf-871d04755da4
- CA3 (Hippocampus) (v11.1) 72f4d9d3-6a14-4b68-8924-bcc01a977f07
- Area OP5 (Frontal Operculum) (v2.0) d9fc6c97-7a2c-4620-aa0f-7ffc9e410918
- Area OP6 (Frontal Operculum) (v2.0) ee72c2a3-bc04-4853-b6db-cb9ecdd247b4
- Area OP7 (Frontal Operculum) (v2.0) ed0b0aee-4260-4d4d-b84b-63a718f24f8d
- Area a29 (retrosplenial) (v11.0) 64845be7-6bfd-4428-a5a5-b61de3d06689
- Area a30 (retrosplenial) (v11.0) 78e278a4-9c9f-4122-baea-102d02cdefc3
- Area i29 (retrosplenial) (v11.0) 563b99ec-6fe3-4b31-bafa-41eaeaa7710c
- Area i30 (retrosplenial) (v11.0) afd7389c-299c-476a-9953-a15750b33ffc
- Area p29 (retrosplenial) (v11.0) 2980bbca-354d-4b9f-b198-59c94545dfdd
- Area p30 (retrosplenial) (v11.0) 40b7a5dd-9c88-47cf-91a2-061f7d3f7559
- HC-Parasubiculum (Hippocampus) (v13.0) e7ebf0c3-a5e8-4382-8447-88714bdcca59
- HC-Presubiculum (Hippocampus) (v13.0) fe24e44e-aef4-4fd2-bf84-16c2d7a8913e
- HC-Prosubiculum (Hippocampus) (v13.0) 11250154-4be9-4024-a12a-61a104cbffb9
- HC-Transsubiculum (Hippocampus) (v13.0) 1de506d5-c303-44b7-a44d-a908dd6804f7

Another recent development is the release of more highly detailed 3D maps of cytoarchitectonic regions for the BigBrain template (Figure 19 right). These maps correspond directly to probabilistic maps of Julich-Brain, but represent a precise delineation performed directly in the BigBrain model using a novel workflow based on Deep Learning, across all histological sections. For example, for area hOc1, delineations in 2,461 histological sections have been computed and reconstructed into a full 3D map. Besides a set of interpolated maps, detailed maps are now available for areas hOc1, hOc2, hOc3v, hOc5, and LGB-lam1 up to LGB-lam6 of the metathalamus[10]. A map of the entorhinal cortex is already under curation and will become available soon.

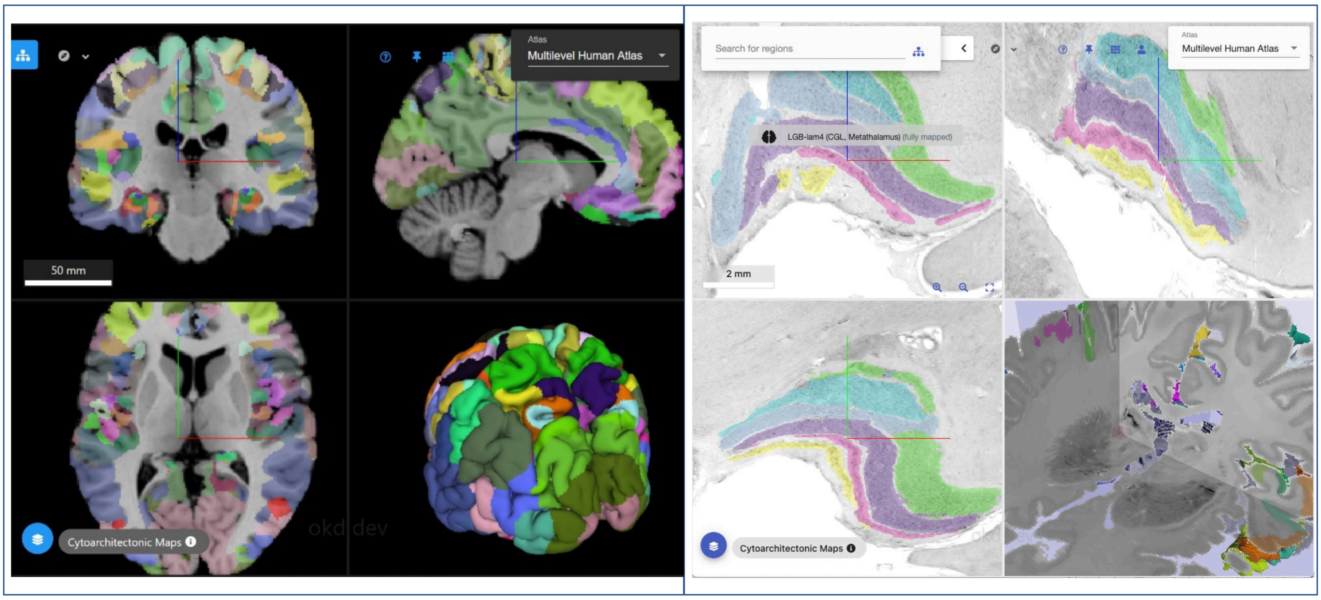

Figure 19: New cytoarchitectonic maps available in the EBRAINS human brain atlas.

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[10] https://kg.ebrains.eu/search/instances/Dataset/d0c36f4a-91a8-4885-880d-f2896f5c54cf

Left: Julich-Brain maximum probability cytoarchitectonic maps with many new region definitions and full coverage of the cortex (https://atlases.ebrains.eu/viewer/saneUrl/colin_cytomap). Right: New microscopic resolution 3D maps in BigBrain; here: Maps of areas LGB-lam1 - LGB-lam6 of the methatalamus (e.g., LGB-lam1: https://atlases.ebrains.eu/viewer/saneUrl/bigbrain_lgblam1).

Furthermore, the diffusion MRI dataset of the Human Connectome Project has been used to compute the largest map to date of reproducible short fibre bundles (less than 8 cm) of the human brain (Labra AN et al., 2019) (P2540), see Figure 20. This map results from a framework aligning subjects using a diffeomorphic procedure forcing the match of the main cortical sulci. This is a key feature to align correctly the U-bundles, which circumvent the cortical folds. This sulcus-based alignment includes the nonlinear ICBM T1-weighted MRI average in order to drive all the subject's tractograms (the set of dMRI-based virtual fibres) to the standard ICBM space. Then, a two-level clustering (intrasubject and intersubject) was performed on the tractograms to define the most reproducible bundles across subjects. A post registration with freesurfer space leads to label each bundle according to the nomenclature of the Desikan-Killiany atlas. As the previous bundle maps in EBRAINS, probabilistic maps of each bundle are accessible together with an aggregated map providing a parcellation of superficial white matter defined from a maximum probability principle.

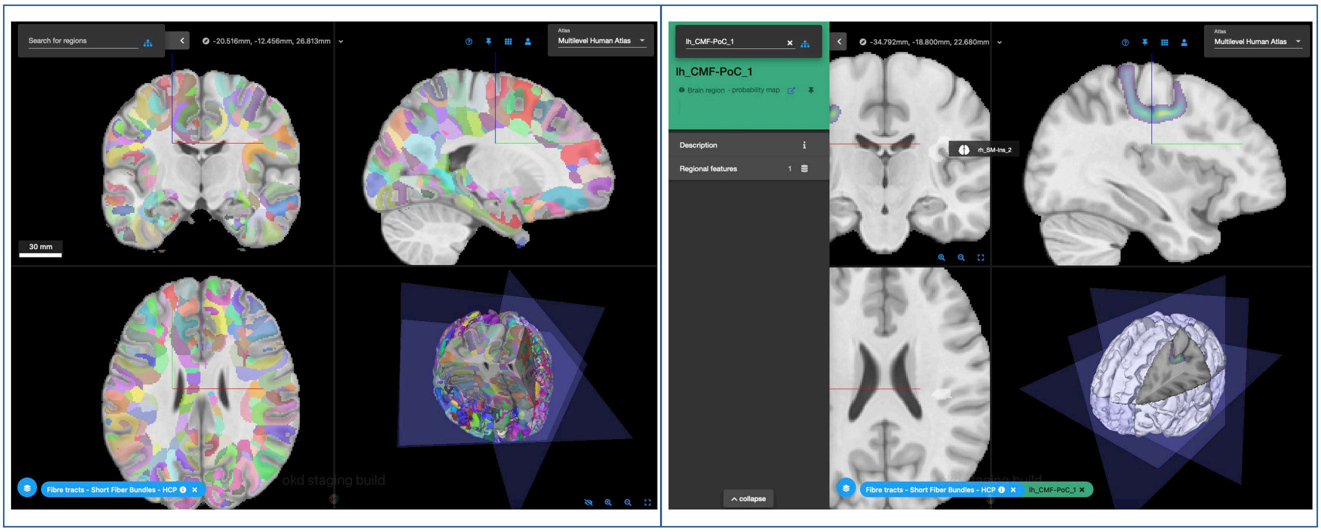


Figure 20: The new maps of short white matter bundles based on the HCP dataset.

The maps of short white matter bundles are available in the Interactive Atlas Viewer: https://atlases.ebrains.eu/viewer/saneUrl/hcp_shortbundles.

The Waxholm Space Rat Brain atlas and the Allen Mouse Brain atlas are now also available via the EBRAINS Interactive Atlas Viewer with optional display of different atlas versions, hierarchical overview of delineated structures, and links to EBRAINS datasets connected to the atlases. Updated atlas versions with hierarchically organised delineations, are now also available in the NUTIL analysis tool. The hierarchical organisation improves data queries and analyses, allowing users to specify regions or selected substructures.

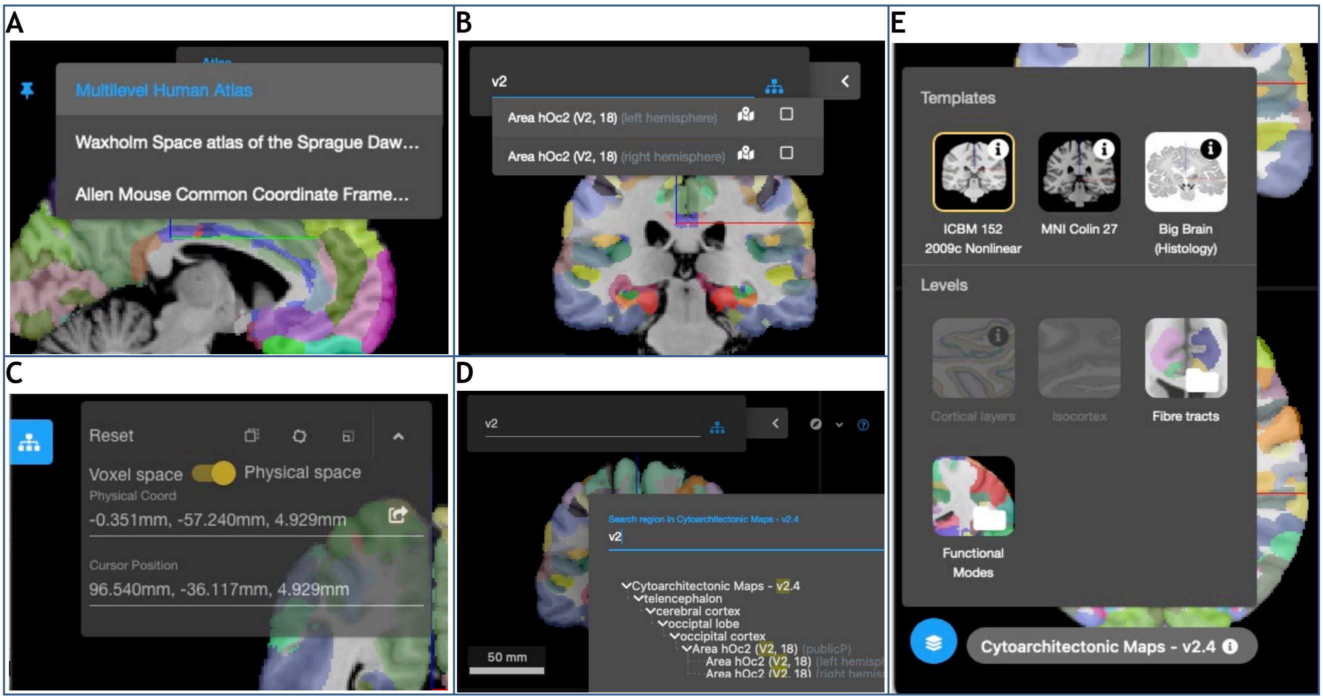
3.2 New release of Interactive Atlas Viewer

The latest release of the 3D Interactive Atlas Viewer described in Section 2.2.1 has received a major update of the user interface that incorporates several improvements, suggested by users, and implements a range of new functionalities. Most importantly, it supports the concept of a multilevel atlas more explicitly by allowing one selected atlas to provide different complementary maps in multiple reference spaces, each with possibly multiple granularities and versions.

The new features include an improved search box for finding, selecting and navigating to brain regions (Figure 21B) that can be expanded into a full searchable region hierarchy browser (Figure 21D), a new layer browser which provides navigation between different reference template spaces and maps offered by a multilevel atlas (Figure 21E), and a coordinate navigation tool for direct manipulation of the view in physical and voxel coordinates, reset of rotation/zoom/pan, and sharing the current scene (Figure 21C).

Regions can now simply be selected by double-clicking a location in any of the image views or using the region search mentioned above. On selection, the viewer will automatically load the corresponding probability map if available and open a side panel with rich information about the selected region (Figure 22). The region side panel includes:

- Region name and location, allowing to navigate to the region centre.
- Information about the corresponding dataset and link to its Knowledge Graph page, if available[11].
- Access to maps of the same region defined in other reference spaces, if available, which can provide a direct link across scales.
- A list of datasets linked to this region ("regional features").
- Connectivity information from this region to other regions.



Physical Coord
-0.351mm, -57.240mm, 4.929mm

Cursor Position
96.540mm, -36.117mm, 4.929mm

Figure 21: Basic controls in the EBRAINS Interactive Atlas Viewer.

A: Atlas selector, B: Quick search for finding/selecting/navigating to brain regions of the current map; C: Coordinate navigator with option for persistent URL sharing; D: Extended region hierarchy browser, accessible from B; E: Layer browser for exploring the different reference template spaces and parcellations of a multilevel atlas. New functionalities of the Interactive Atlas Viewer can be explored here: https://atlases.ebrains.eu/viewer/.

Furthermore, the side panel allows to explore more types of regional data features than previous versions of the atlas viewer and provides significantly improved data exploration panels for certain data modalities. These include:

- **iEEG recordings** (Figure 23). It is now possible to find iEEG recordings close to a selected brain region as a regional data feature and interact with them. This includes selection of different electrodes and individual contact points, and display of their locations in the 3D view, which is switched to transparent mode. The actual recordings will be directly accessible via the viewer as soon as EBRAINS releases the GDPR-compliant authentication layer.
- **Transmitter receptor densities** (Figure 24). A redesigned side panel for exploration of receptor transmitter densities allows to interact with the fingerprint to find cortical profiles and sample autoradiographs of specific receptors.
- **fMRI measurements.** Among the regional data features the viewer now also lists fMRI scans with strong activations in the selected regions. This is currently restricted to a list of scans from the

---

[11] For some maps, especially the Julich-Brain cytoarchitectonic maps, each area is a separate dataset with its own publication and information.

Individual Brain Charting project. The actual files will be directly accessible from the Knowledge Graph via the viewer as soon as EBRAINS releases the GDPR-compliant authentication layer.

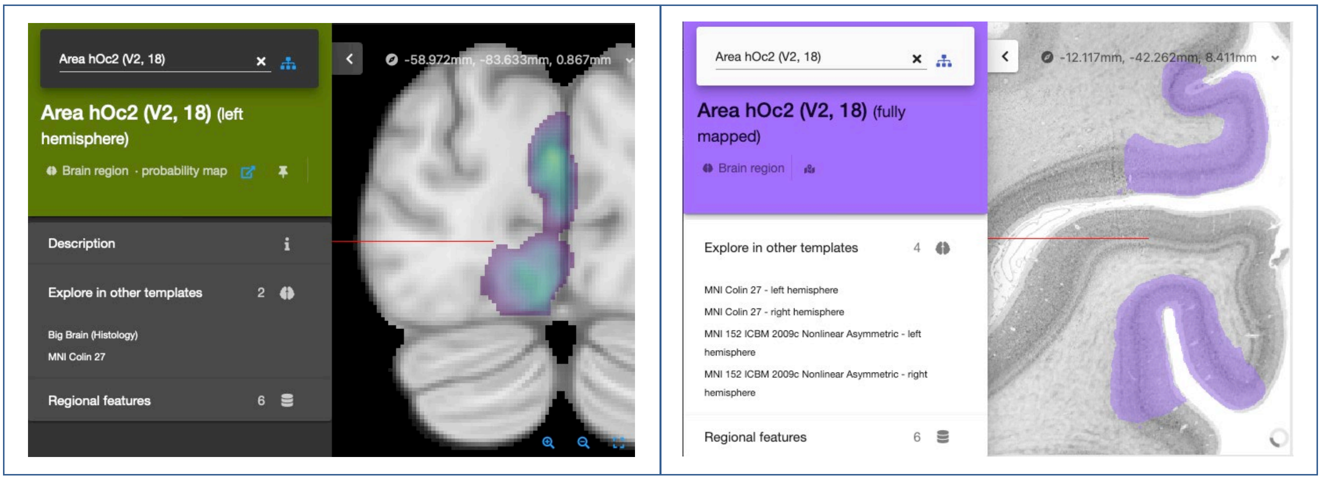


Figure 22: Side panel with information about a selected region and links across scales.

Left: Selecting Area V2 from Julich-Brain probabilistic cytoarchitectonic maps in MNI space loads the corresponding probability map, and provides links to other reference spaces, data features, and connectivity information (https://atlases.ebrains.eu/viewer/saneUrl/icbm_hoc2). Right: The cross-linked high-resolution map of V2 in the BigBrain reference space, which defines the same region at microscopic resolution, giving access to cellular level information (https://atlases.ebrains.eu/viewer/saneUrl/bigbrain_hoc2).

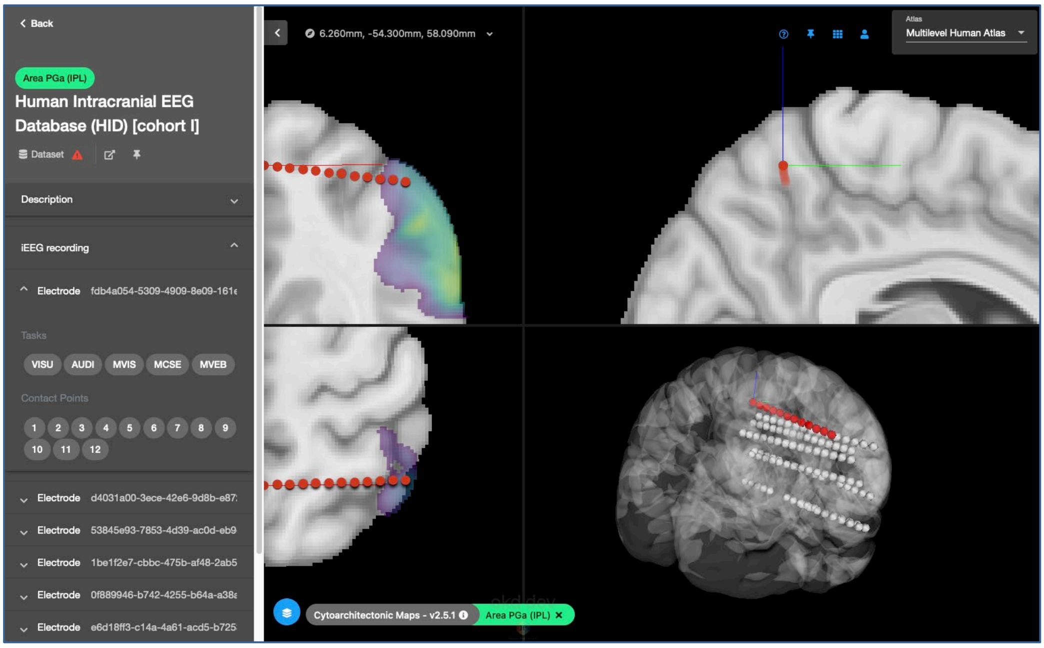
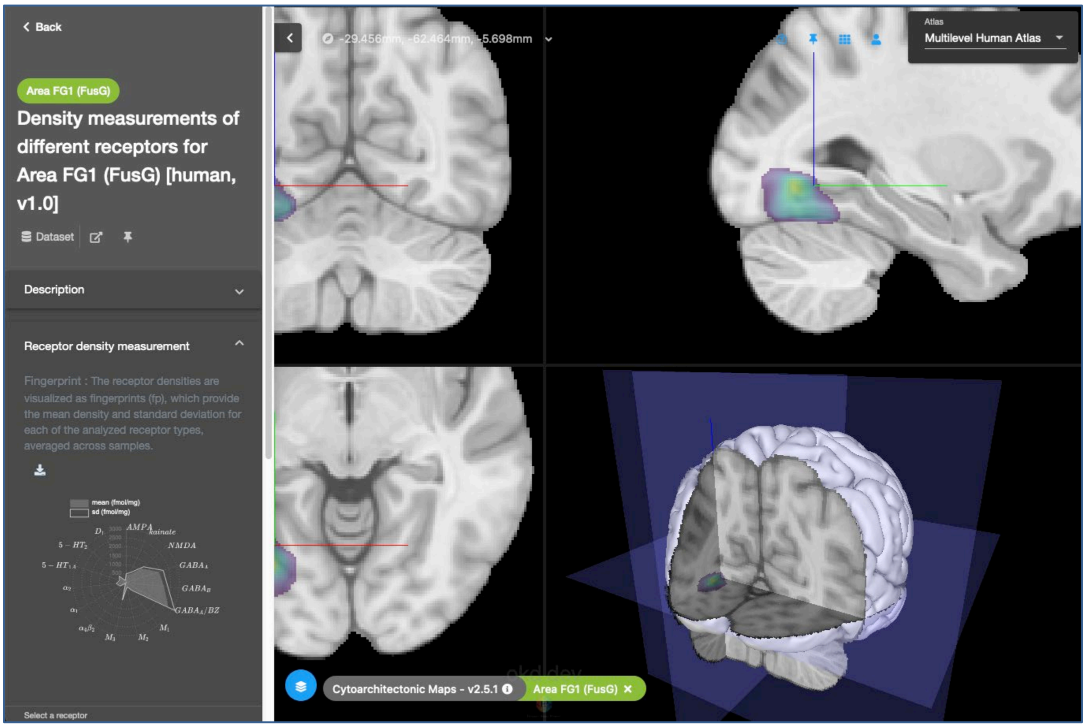


Figure 23: Atlas viewer side panel for exploration of iEEG recordings in the selected region.

The iEEG recordings are available in the Interactive Atlas Viewer here https://atlases.ebrains.eu/viewer/saneUrl/icbm_pga by opening related datasets in the side panel.



Density measurements of different receptors for Area FG1 (FusG) [human, v1.0]

Fingerprint : The receptor densities are visualized as fingerprints (fp), which provide the mean density and standard deviation for each of the analyzed receptor types, averaged across samples.

Figure 24: Atlas viewer panel for exploring receptor densities of a particular brain region.

The panel allows interactive selection of transmitter receptor types from the fingerprint, to get access to corresponding cortical profiles and example autoradiographs. The receptor data are available in the Interactive Atlas Viewer here https://atlases.ebrains.eu/viewer/saneUrl/icbm_fg1 by opening related datasets in the side panel.

3.3 Prototype Python atlas client

The prototype of a Python client for accessing EBRAINS atlases has been developed. This client gives direct programmatic access to typical patterns of interaction with EBRAINS atlases, in the prototype version focusing on parts of the multilevel human atlas. It supports the multilevel character of this atlas as described in Section 2.1.1. The functionality of the Python client matches common actions known from browsing the Interactive Atlas Viewer: selecting a parcellation, browsing and searching brain region hierarchies, downloading maps, selecting regions, and requesting manifold information and features associated with brain regions (see 2.2.1). The atlas client has been used for implementing a direct link to simulation, as explained, and demonstrated for the Showcase 1 in WP 1.

A key feature of the atlas client is a streamlined implementation of performing data queries for selected brain regions, which gives access to multimodal regional “data features”. The client implements a hierarchy of features, which unifies handling of spatial data features (linked to atlases via coordinates; like contact points of physiological electrodes), regional data features (linked to atlases via brain region specifications, like cell densities or neurotransmitter distributions) and global data features (linked to atlases via the whole brain or parcellation, like connectivity matrices or activation maps). As a result, all forms of data features can be queried using a single function (“query_data()”) which takes as an argument a specification of the desired data modality, and respects the current selections made in the atlas. For the current prototype, available data features include neurotransmitter densities, regional connectivity profiles, connectivity matrices, gene expressions, and spatial properties of brain regions.

The Python client hides much of the complexity that would be required to interact with the individual online services. By encapsulating many aspects of interacting with different maps and reference templates spaces, it also minimises common risks like misinterpretation of coordinates from different reference spaces, or utilisation of inconsistent versions of parcellation maps. It aims to provide a safe way of using maps defined across multiple spatial scales. When a query is performed, the client stores downloaded items in a local cache to speed up later queries.

4. Future features of EBRAINS atlas services

4.1 Atlases of different species

- The human atlas will be extended to support the commonly used freesurfer surface space, in order to complement the currently used MNI volumetric spaces. This feature will become available in November 2021 (M20).
- The complementary maps included in the human atlas will be more tightly integrated with each other by exploiting region overlaps, hierarchical relationships and proximity information. This feature will be released in 2022.
- The human atlas will be significantly extended by more regional data features from high-resolution modalities. A release of neuronal cell distributions in a large portion of available atlas regions is scheduled for October 2021 (M19).
- A finalised version 4 of the WHS rat brain atlas will be released in September 2021 (M18), with 45 additional detailed delineations in the cerebral cortex, basal ganglia, and thalamus (Figure 25).

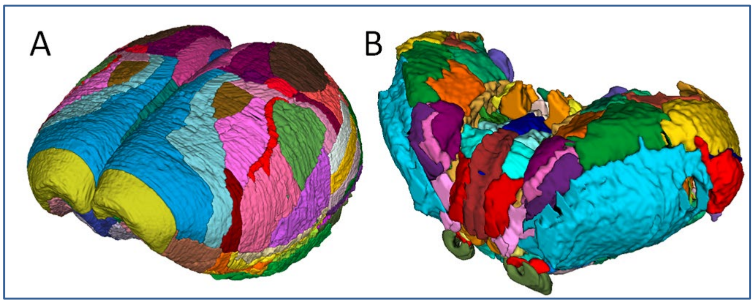


Figure 25: Waxholm Space Rat Brain atlas version 4beta.

(A) Detailed delineations of the cerebral cortex. (B) Detailed delineations of the basal ganglia and thalamus.

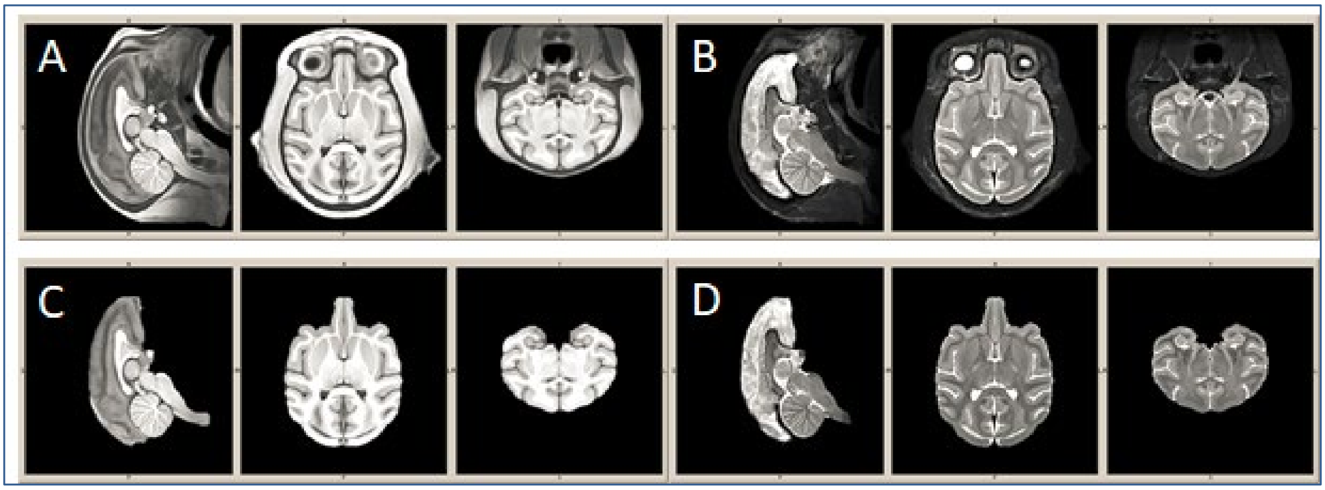


Figure 26: Anatomical template of the macaque monkeys.

Whole brain T1 (A) and T2 (B) images, and skull stripped brain T1 (C) and T2 (D) images. The T1 templates (A, C) are the average brain of 38 monkeys, while the T2 templates (B, D) are the average brain of a sub-set of the 38 monkeys for which both T1 and T2 good images were recorded.

- EBRAINS atlases will be extended by a new Non-Human Primate Atlas (NHP). The atlas will be based on imaging modalities of magnetic resonance imaging (MRI) - T1 and T2 weighted as well as diffusion weighted imaging - and computed tomography (CT) of more than 30 macaque monkey brains. A new monkey template has already been created as a multi-subject average (Figure 26), using the multi-brain toolbox for SPM12[12] which has shown superior results to a previous model based on ANTS. The template will serve as anchorage for multi-resolution spatial and temporal experimental data, as well as for maps of distinct cyto- and receptor architectonically identified areas such as those recently published for the macaque motor and premotor cortex (Rapan L et al., 2020) (P2623). A first version of the NHP atlas will become available in September 2021 (M18).

4.2 Exploring atlases

- The 3D Interactive Atlas Viewer will support viewing of local Nifti files and point sets in superimposition with the reference atlas volumes and maps. This feature will be released in September 2021 (M18).
- The 3D viewport of the Interactive Atlas Viewer will be extended to support customised slice views (to be released in September 2021; M18). This will enable basic "virtual dissections" of the BigBrain volume.
- The Interactive Atlas Viewer will support to enter, store, and share 3D landmarks with descriptions. This will allow to "bookmark" brain locations and share them with collaborators. The landmarks can be re-used in the VoluBA tool for spatial anchoring. This feature will be released in May 2021 (M14).
- An image service for efficient streaming of large volumetric image data registered in the EBRAINS Knowledge Graph to the atlas viewers will be released in March 2022 (M24).
- EBRAINS atlas services will expose strategic datasets via the DataLad ecosystem (https://www.datalad.org/) to allow well-structured and versioned downloads. This feature will be released before March 2023. A beta release will appear in 2022.

4.3 Integration of multimodal data to atlases

- The QuickNII and VisuAlign tools for integrating 2D sections to reference atlases will become available in 2022 - 2023 as web tools. This will lower the threshold for uptake of the tool by the broader community.
- The experimental plugin for cortical depth driven nonlinear alignment of partial cortical volumes to BigBrain will become a core functionality of VoluBA. This feature will be released in 2022.
- The HBP Morphology Viewer[13] is a web-based tool to inspect, edit and align neuronal morphologies. It was developed during the ramp-up phase of the Human Brain Project for processing files in the *Neurolucida* format, for which the viewer implements a parser. The morphology viewer is now part of a pilot neuronal registration pipeline (Figure 27) that involves the interplay between three web-based components:

  1) a database component that supplies the neuron
  2) the morphology viewer
  3) an atlas registration tool for morphologies

  In the pilot implementation, component (1) is the user's local computer, and component (3) is the SBA Composer[14], the 3D version of the Scalable Brain Atlas. The spatial transformation resulting from the process can be submitted to the EBRAINS curation teams together with neuron morphology files. EBRAINS is currently developing a bidirectional interface with the morphology viewer to further facilitate this process. The Neuron Morphology Viewer will be integrated into the EBRAINS Platform. Here, EBRAINS will take the role of a database component (using the new

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[12] https://github.com/WTCN-computational-anatomy-group/diffeo-segment

[13] http://neuroinformatics.nl/HBP/morphology-viewer

[14] http://sba.incf.org/composer

SANDS scheme for spatial metadata), visualisation engine (using the 3D Interactive Atlas Viewer). To this end, the within-browser communication protocol used by the Morphology Viewer will be transferred to the EBRAINS Interactive Atlas Viewer. The advantage of using EBRAINS over SBA Composer will be that neurons can be displayed in a high-resolution atlas and alongside other data within the EBRAINS ecosystem. After completing the EBRAINS integration, we will decide whether to rely on SBA Composer or EBRAINS to interactively register neurons into the atlas (Figure 28). This integration will become available as a beta release in 2022, and as stable release in 2023.

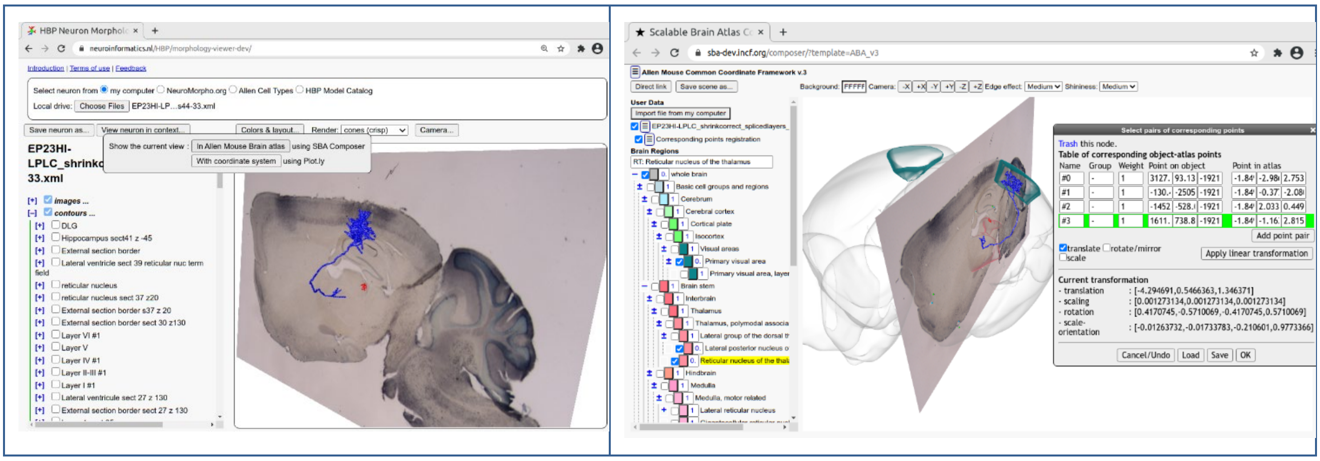

Figure 27: Current pilot pipeline for alignment of neuron morphologies.

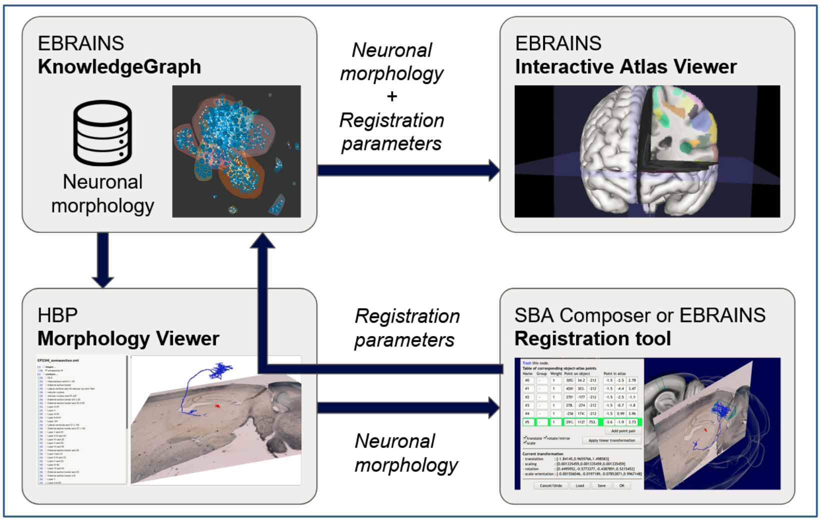

Figure 28: Planned architecture for integrating neuron morphology alignment with EBRAINS.

4.4 Tools for analysing data using EBRAINS atlases

- An interface of EBRAINS atlas services with the popular nipy[15] library for analysis of neuroimaging data will be developed. This interface will be released before March 2023, and a beta version in 2022.
- A new *neuroimaging toolbox* will be integrated with the EBRAINS atlas viewer and Python client, which enables probabilistic assignment of neuroimaging signals to EBRAINS atlas parcellations, similar to the popular *SPM anatomy toolbox*. The toolbox will be released in 2022.
- The QUINT workflow will be extended to include new features for easier use, improved analysis, and integration with the EBRAINS Knowledge Graph: The *Nutil* tool will become available as a web service in early 2023. Direct links from the Knowledge Graph Dataset cards to viewing of the

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[15] http://nipy.org/

output from the QUINT workflow will be provided. All tools will be updated with newest atlas versions.

- Based on the *BayesSuites*[16] software, the Python library *neurogenpy* will be integrated with EBRAINS, which allows analysis of gene expression data and learning of gene regulatory networks. The integration will include an interface with the EBRAINS Interactive Atlas Viewer to run a limited form of the network learning process interactively, and export learned networks in a well-defined metadata format compatible with the EBRAINS Knowledge Graph. This toolchain will be released in early 2023.

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6. Annex

6.1 Listing of known problems, possible risks and delays

- A remaining challenge for rodent brain atlasing is to increase the visibility and accessibility of the data that have been integrated in the mouse and rat brain atlases. The ongoing openMINDS implementation and atlas tools improvements aims to solve this problem by the end of 2021.
- Development of the EBRAINS image service suffered some delays due to hiring of personnel. These delays have now been compensated for with the internal release of the service for EBRAINS application development. Downstream analysis tools depending on the image service – including ilastik, QUINT workflow, and atlas viewers - are now catching up on the delay.
- Spatial integration of datasets to brain atlases, especially for the highly variable human brain, requires efforts depending on the nature of the data. These range from routine tasks that can be covered by data curation teams, to highly complex problems like part-to-whole alignment across human subjects which are still open research problems. Therefore, the fraction of datasets shared via EBRAINS that are appropriately linked to atlas regions is difficult to estimate in advance. By implementing and employing solutions for several cases, we aim to spatially integrate a comprehensive set of data features.
- Identification of strategic cross-species homologies of brain areas in non-human primates and humans (Milestone 4.2) requires careful review of current knowledge in the field and intense scientific interactions, which have been impeded by the Covid-19 pandemic. Integration of such homologies as supporting metadata into the digital atlas system will start with a limited initial set which will be continuously extended.
- Some atlas services, in particular some of our online analysis tools, are very useful for ad-hoc applications using user-supplied data in addition to datasets which are already shared via EBRAINS. To achieve compliance with legal and ethical regulations, implementation of ad-hoc ingestion workflows for user-supplied datasets is closely supervised by our data protection officers and legal advisers. This process can sometimes lead to delays in releasing online workflows.