Atlas-based data integration for mapping the connections and architecture of the brain

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Detailed knowledge about the neural connections among regions of the brain is key for advancing our understanding of normal brain function and changes that occur under ageing and disease. Researchers employ a range of experimental techniques to map connections at different levels of granularity in rodent animal models, but the results are often challenging to compare and integrate. Three-dimensional reference atlases of the brain provide new opportunities for cumulating, integrating, and reinterpreting research findings across studies. We review approaches for integrating data describing neural connections and other modalities in rodent brain atlases and discuss how atlas-based workflows can facilitate brain-wide analyses of neural network organization in relation to other facets of neuroarchitecture.

Introduction

The brain is composed of vast numbers of neurons, glia, and vasculature, encased within a solid skull. It processes and stores information, generates memories, thoughts and ideas, performs planning, and effectuates a wide range of behaviours. Dendrites and axons allow neurons to transmit signals across shorter or longer distances, and axons profusely branch into terminal fields with multiple synaptic contacts to other neurons. The functions performed by neurons are to a high degree determined by their connections with other neurons within and across brain regions.

Large ensembles of widely distributed neurons make up complex neural networks. The networks are highly organized, typically with different cell types distributed in layers or clusters. Within the network, populations of neurons exert specific excitatory, inhibitory or modulatory influences on other parts of the network, and variations in the strengths and spatial distributions of the connections, including specific patterns of divergence and converge, influence how the network subserves its functions. Overall, knowledge about the organization of the networks – the wiring patterns of the brain – is critical for understanding normal brain function, and is typically embedded in network models aimed at elucidating and studying a variety of brain functions (1). Insight into the detailed organization of wiring patterns is also key to understanding and treating brain disorders. One example is the use of knowledge about the wiring of deep brain structures (Fig. 1AB) for treatment of neurological disease, e.g., the use of electrical stimulation targeting the subthalamic nucleus or specific parts of the thalamus for ameliorating symptoms of Parkinson's disease and medication-resistant tremor (2).

While the patterns of neural wiring direct how neural signals are distributed through a network, the functional characteristics of a network also depend on the physiological and neurochemical properties of neurons, their detailed local cellular relationships to other neurons (micro-circuitry) and to supporting cells with sustaining or modulatory functions. Comprehensive knowledge about how the brain exerts its functions thus requires integration of knowledge about all these features. We argue that recently introduced three-dimensional (3D) digital brain atlases (3-6) offer new opportunities for extensive data integration aimed at improving our understanding of the organization of the brain. These integration efforts are accelerated by the use of tools for registration of heterogeneous data types to the atlases, in combination with computerized workflows for subsequent automated analyses of large data collections.

Focusing on the rodent brain as a model system for basic neuroscience, we review approaches for the mapping of neural connections and atlas-based solutions for integrating and analysing data, before discussing future directions for advancing the field.

Mapping brain connections

A variety of techniques are available for mapping of neural connections at different levels of granularity. The overall trajectories of fibre bundles in the brain can be visualized with myelin staining (e.g. 7, 8; Fig. 2B) or polarized light imaging in histological sections (e.g. 9; Fig. 2B), and whole brain diffusion MRI methods (e.g. 8. 10, 11; Fig. 2B). The "neuron-by-neuron" connections are mapped with use of high-resolution microscopic techniques allowing imaging of tracer-filled individual neurons (e.g. 12, 13), or by use of serial electron microscopy visualization of cellular and synapse ultrastructure (e.g. 14). The current foundation for whole- brain mapping of neuronal connections is, however, provided by invasive tract-tracing experiments in wild-type and transgenic animals (Figs. 1, 2; 15, 16). These methods are highly suitable for describing connections at the level of groups of neurons, demonstrating patterns that are persistent and reproducible among individuals and useful for inferring functional properties and disease related changes (for a discussion of different levels of connectivity analysis, see, 17).

In classical tract-tracing experiments, a tracer substance is deposited in a specific location in the brain and taken up by groups of neurons (Fig. 1C-E). The tracer is transported along the axons of the neurons, either anterogradely from neuronal cell bodies to their axonal terminal fields, or retrogradely from axonal terminal fields to the neurons of origin, or in both directions (15, 16). Depending on the tracer employed, some of the morphologies of the labelled neurons are revealed, or the tracer is transported across synapses, allowing identification of pre- and postsynaptic connections in a network (16). New genetic animal constructs have also opened for advanced cell-type specific tracing paradigms, with genetically controlled expression of signals (18).

A key methodological innovation for the tract-tracing methods has been serial two-photon tomography (19). By allowing block-face acquisition of high-resolution microscopic images, this technology has been successfully utilized by the Allen Institute to generate large volumes of microscopic 3D tract-tracing image data

showing brain wide connections in the mouse brain (5, 18). Using a similar approach, the MouseLight project of Janelia Research Campus has created high-resolution volumetric reconstructions of individual axonal trajectories across entire mouse brains (20, 21).

Atlases for brain-wide mapping of connections and related features

Traditionally, experimental tract tracing studies have focussed on one or a few brain regions at a time, yielding precise information about the connections among a few selected regions of interest. Extensive literature mining efforts have aggregated information from available publications into databases, to attain a more complete understanding of the connections between brain regions (22, 23). However, these valuable resources are limited by the diversity of the methods used, the variable levels of precision in the reporting of brain location, and the lack of access to the underlying data (17, 24). Regarding reporting of the anatomical location of observations, a recent analysis of practices and precision from 120 different rodent brain experimental studies revealed substantial differences in the research design, interpretation of results, and reproducibility among reports (25). The main challenges were related to the use of different parcellation schemes and the lack of precision in the reporting of how observations would map onto a given anatomical scheme. While atlases for mouse and rat brains (26-28) have assisted researchers for decades in assigning location to their observations, their utility is limited in that they are two-dimensional and lack efficient and standardized tools for the registration of observations to the atlases. These limitations have been overcome with a new generation of open access 3D atlases, which integrate information from multiple anatomical parcellation schemes and have powerful tools for the registration of data to atlases and atlas based analysis.

For the mouse brain, the atlases developed by the Allen Institute for Brain Science have become widely used resources. The most recent version is defined in a high-resolution image volume constructed by interpolation of serial two-photon tomography (STPT) images from 1675 adult mice (the Allen Mouse Brain Common Coordinate Framework, CCFv3). In this population-averaged image volume many anatomical delineations were defined using information from a large body of multimodal image data registered to the CCFv3 template (6). The underlying images included the collection of STPT data created for the Allen mouse brain connectivity

atlas (5). Additional images were readily integrated into the atlas volume using the same high-throughput imaging and informatics pipeline, as achieved with the inclusion of more than 1,000 STPT image volumes from tract-tracing experiments conducted in transgenic Cre-dependent mice (17).

For the rat brain, the Waxholm Space (WHS) atlas is now available in a version 4 with brain-wide parcellation (RRID**:** SCR_017124; https://nitrc.org). This atlas is based on a single high-resolution *ex vivo* structural and diffusion magnetic resonance imaging volume, in which brain regions have been identified and delineated by manual interpretation of the underlying MRI data, enriched with multiple microscopic image data showing different facets of the neuroarchitecture (4, 29, 30; see also, Fig. 2). The atlas has seen broad interest as reflected in close to 25,000 downloads, more than 300 citations, and inclusion in several services or products (e.g., EBRAINS research infrastructure, https://ebrains.eu, and the Neuroinfo software of MBF Bioscience). To facilitate comparisons of different atlas parcellation schemes, seven versions of the Paxinos and Watson rat brain atlases, and four versions of the Swanson rat brain atlas, were registered to the WHS rat brain atlas (31, 32).

Atlas-based data integration and analysis

The 3D reference atlas spaces provided by the Allen Mouse Brain Common Coordinate Framework (CCFv3 and earlier versions), and the WHS rat brain atlas are useful frameworks for integrating heterogeneous data originating from different researchers and research projects. Recently introduced tools and workflows designed for use with the mouse and rat atlases support a 3-step process for data integration and analysis (Fig. 3): 1) registration of images to atlas, 2) sharing of registered images with viewing and navigation of images in atlas space, 3) extraction of features from the images followed by quantification of the distribution of features within and across brain structures. Below, we review the principles and practical implementation of the three steps, taking a starting point in the EBRAINS Atlases services (https://ebrains.eu/services/atlases) for the mouse and rat brain and the EBRAINS Data and Knowledge services for data sharing and access (https://ebrains.eu/services/data-and-knowledge).

Registration of images to atlas

Data integration requires that the data are made comparable, as if data of different origin were part of a single data set. Thus, the registration of data of different origin to the same atlas framework is a key step towards integration. In EBRAINS, series of 2D histological images of mouse or rat brains are spatially registered to the atlases by an initial landmark-based anchoring of sections, followed by a non-linear adjustment. The EBRAINS tools supporting this step are the QuickNII tool for affine registration (RRID: SCR_016854; 33), and the VisuAlign tool for non-linear registration adjustments (RRID: SCR_017978). The output of the registration process is referred to as *spatial metadata*: a set of anchoring vectors and deformation fields that together define the transformations between the image data and the atlas. A range of other registration tools are available, with some having been developed for particular use cases such as analysing electrode tracts (34, 35), and others suitable for many data modalities (36).

Sharing, viewing, and navigating images in atlas space

In addition to having spatial metadata, data are integrable only if they are properly annotated with metadata and other structured information that help us understand the data. Furthermore, the data will have to be discoverable and accessible. The approach taken by the EBRAINS Share data service (https://ebrains.eu/service/share-data) is to provide procedures for annotation of the data with metadata according to a metadata standard, and to provide descriptions and other information required to make the data interpretable and reusable. Following a curation of the metadata, the data are stored in the EBRAINS data repository, whereas the metadata are ingested in a knowledge graph which makes the data findable through a Search user interface (https://search.kg.ebrains.eu), or a programmatic access route (https://ebrains.eu/service/find-data/). A search points the user to Data cards with information about the data, access to the data sets, and also links to a virtual microscopy viewer for inspection of the image data integrated in the atlas.

With use of the EBRAINS tools, a broad range of data generated with different methods and in different animals have been registered to the atlases. Figure 2 shows examples of histological images registered to the WHS rat brain atlas v4. In the thalamus, as the selected region of interest, data on specific connections of the primary somatosensory cortex are available together with images showing bundles of

axons and their orientation with myelin staining and polarized light imaging. Figure 2C demonstrates some of the basic functionalities of the virtual microscopy viewer that is available via the Data cards. The viewer supports web-based pan-and-zoom of high-resolution images with overlays of the atlas parcellation map generated by a volumetric atlas slicer. The user can inspect the images at cellular resolution and observe brain regions, names, and boundaries and annotate points of interest to extract atlas coordinates.

Feature extraction, quantification, and distribution

Finally, features from the images can be extracted, sorted by brain region, and displayed and further analysed in 3D. To this end, various approaches are employed as exemplified in the EBRAINS workflow for automation of several of the steps (37, 38). This workflow consists of a registration tool, a machine learning based tool for extraction of selected features in the images (Ilastik, 39), and finally a tool for quantifying the features per atlas region (Nutil, 38). The workflow allows the users to customise their analysis in many aspects, including choosing the granularity level of the atlas, defining their own regions of interest, filtrating artefacts, and applying quality control steps. It also allows export of coordinates to other tools for 3D visualization of the distribution of the selected features, from the whole brain or selected regions. Figure 4 shows examples of regional analysis of brain connection features extracted from images registered to the WHS rat brain atlas and the Allen Mouse Brain Common Coordinate Framework.

Figure 4 shows examples of regional analyses of brain connection features extracted from images registered to the WHS rat brain atlas or the Allen Mouse Brain Common Coordinate Framework. The data originate from different research projects and data repositories, but are integrated and made comparable by registration to the same atlas spaces. Specific combinations of tract-tracing data showing terminal fields of axons in the corticopontine projection system have been selected for analysis of topographical organization (Fig. 4A-D), and identification of changes in topography resulting from lack of specific gene expression (Fig. 4E,F). Furthermore, tract-tracing data showing corticopontine projections from large groups of cortical neurons have been combined with 3-D reconstructions showing individual neurons and their extensive branching patterns, including branching to multiple target clusters within

the pontine nuclei and elsewhere in the brain stem (Fig. 4G, H), illustrating opportunities for parallel processing and neural circuit complexity.

Conclusion and outlook

Online repositories containing large collections of experimental data integrated in an open access volumetric reference atlas have proven successful for mouse brain research, evident from the impressive data and results provided, e.g., by the Allen Institute and the Janelia Research Campus (5, 18, 20, 21). Open access sharing of standardized data mapped in an appropriate anatomical context make it possible to find and efficiently use new combinations of data, suitable for characterizing and investigating many aspects of brain connections. However, despite the impressive amounts of data presented, attempts to utilize these generous resources may also reveal that data coverage may be insufficient to answer challenging questions, as exemplified in the recent study by Tocco et al. (40), demonstrating that studies of topographic organization in the corticopontine projection require precisely corresponding tracer injections to detect subtle changes occurring in transgenic animals (Fig. 4E,F). Similarly, attempts to compare the individual axonal morphologies in the pontine nuclei (using data from the MouseLight project at Janelia Research Campus) to the terminal fields visualized in tract-tracing experiments (using data from the Allen Mouse Brain Connectivity Atlas) reveal that data mapped to the same atlas allow interesting observations (Fig 4G,H), but also indicate that more data are needed for complete mapping of neural networks. Attempts to find, visualize, and compare such data are also hampered by technical challenges related to lack of tools interoperability. In the rat, large data collections on neural connections are not available, and so far few attempts have been made to systematically map brain-wide connections (24). For these reasons, adding more data and tools will be critical for attaining an increasingly complete overview of the organization of brain connections and other features of rodent brain architecture.

The atlases play a key role in this endeavour. They provide a standardized representation of anatomical location and are embedded in software tools for integration and analysis. Data sharing services, such as delivered by EBRAINS, organize the data, and help standardize the metadata, including metadata about the location of research data from the brain. Through the atlas frameworks, data from individual researchers published in research articles and integrated in the atlases are

made directly comparable to data from large scale mapping efforts such as the Allen Mouse Brain Connectivity Atlas, and the MouseLight project. The new paradigm for research on brain connections and brain architecture in general is to bring the research data into the same reference space, share the data, and prepare the data for systematic reanalysis and reinterpretations of our understanding of the brain. With these new approaches being introduced in neuroscience, literature mining can be supplemented with powerful mining of the data underlying the interpretations included in publications.

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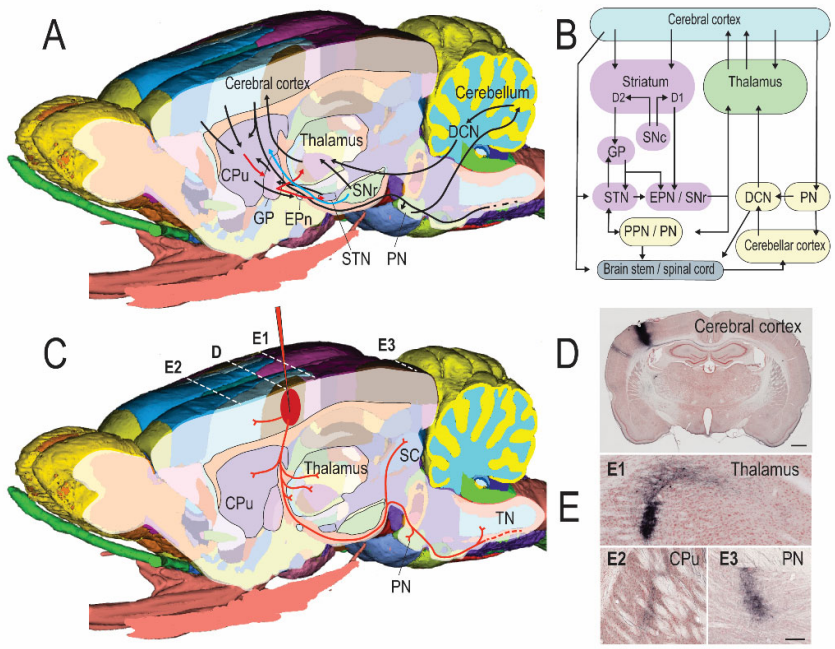

Fig. 1. Wiring patterns in the brain

A,B, Schematic visualizations of basal ganglia and cerebellar neural networks, topologically drawn (**A**) in an oblique slice through the Waxholm Space rat brain atlas (RRID: SCR_017124) and as a box diagram (**B**). **C.** Illustration of tract tracing experiments in which an anterograde tracer is placed in the cerebral cortex, taken up by groups of neurons, and transported along the axons and their branches, to visualize projections to intracortical and subcortical regions. (**D, E**) Microscopic images from a tract tracing experiment (data from 41), in which an axonal tracer (visualized as black labelling) was injected into the primary somatosensory cortex (**D**), giving anterograde labelling of dense axonal plexuses in thalamus (**E1**), caudoputamen (**E2**), and pontine nuclei (**E3**). CPu, caudoputamen; DCB, deep cerebellar nuclei; EPn, entopeduncular nucleus; GP, globus pallidus; SNc, substantia nigra, pars compacta; SNr, substantia nigra, pars reticulata; PN, pontine nuclei; PPN pedunculopontine nucleus; TN, trigeminal nuclei. Scale bars, 1 mm (**D**); 200 µm (**E**).

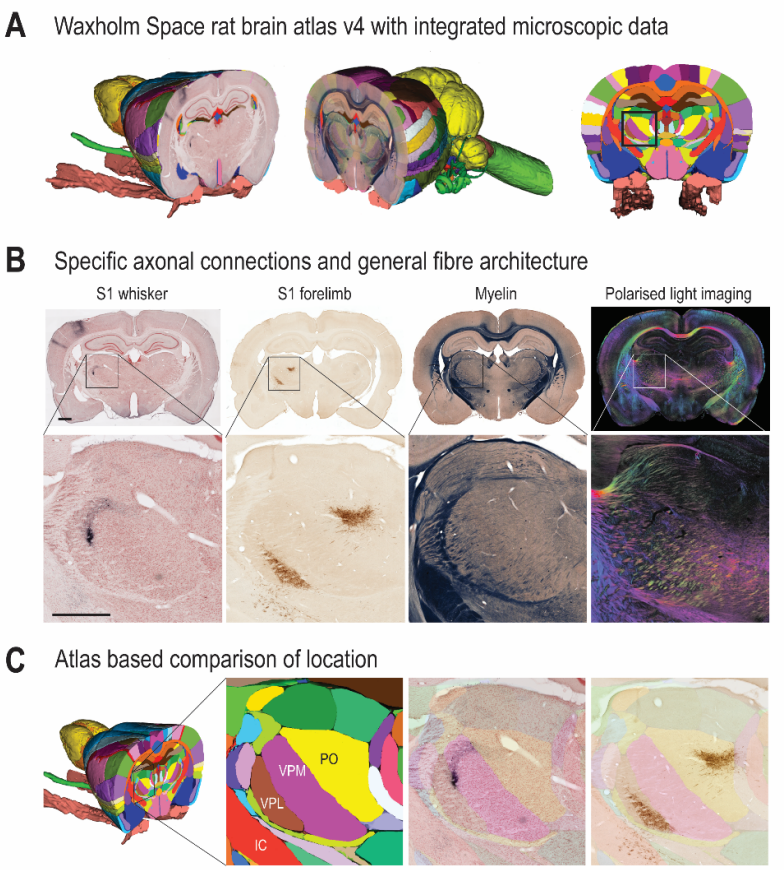

Fig. 2. Waxholm Space rat brain atlas with integrated microscopic data

The Waxholm Space rat brain atlas is enriched with spatially registered microscopic image data allowing comparison across different experiments and data types. (**A**) Illustration of tract tracing and myelin-stained microscopic images of coronal sections registered to the brain at the level of the thalamus, indicated with a frame. (**B**) Overview and details from four different coronal microscopic images taken from the same anteroposterior level of the thalamus, including anterogradely labelled corticothalamic projections from whisker (data from 41) and forelimb (data from 42) representations in the primary somatosensory cortex (S1), tissue fibre orientations visualized by myelin staining (data from 43), and polarized light imaging (data from 44), respectively. (**C**) shows the Waxholm Space rat brain atlas superimposed on the tract tracing image shown in B, providing a starting point for interpreting the spatial location of axonal labelling across subregions of the thalamus. Abbreviations: IC, internal capsule; PO, posterior thalamic nucleus; VPL, ventral posterolateral thalamic nucleus; VPM, ventral posteromedial thalamic nucleus. Scale bars, 1 mm.

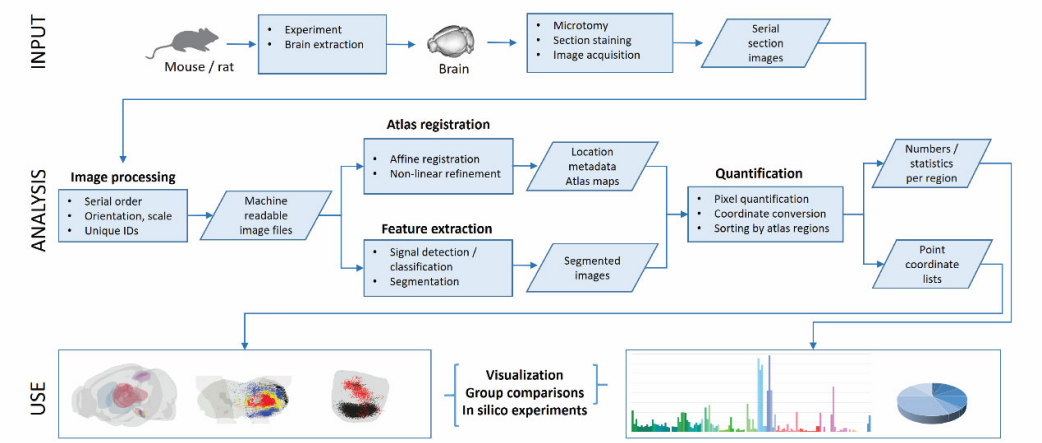

Fig. 3. Workflow for data integration and atlas-based analysis

Diagram showing key steps of a generic workflow for integration of rodent brain experimental data in volumetric brain reference atlases, and atlas based analysis, yielding quantitative data and 3-D point coordinate data sorted to atlas defined brain regions. The input to the workflow is provided by experimental procedures resulting in serial microscopic images of tissue sections showing neural labelling. Pre-processing steps include validation of image order and orientation, assignment of unique serial identifiers, to create machine readable image files. Important parallel analytic steps are the spatial registration of images to a volumetric reference atlas, and extraction of labelling signals from background, providing input combined in (automated) analyses extracting quantitative measures and 3-D point coordinates representing selected labelling features. The workflow output can be visualized and utilized in statistical analyses for characterizing and comparing labelled parameters. Point coordinates representing labelled neuronal elements are suitable for interactive 3-D visualization and exploration of spatial distribution patterns, and hypothesis-driven *in silico* experiments visualizing selected combinations of data. Usage example data are taken from (45-49).

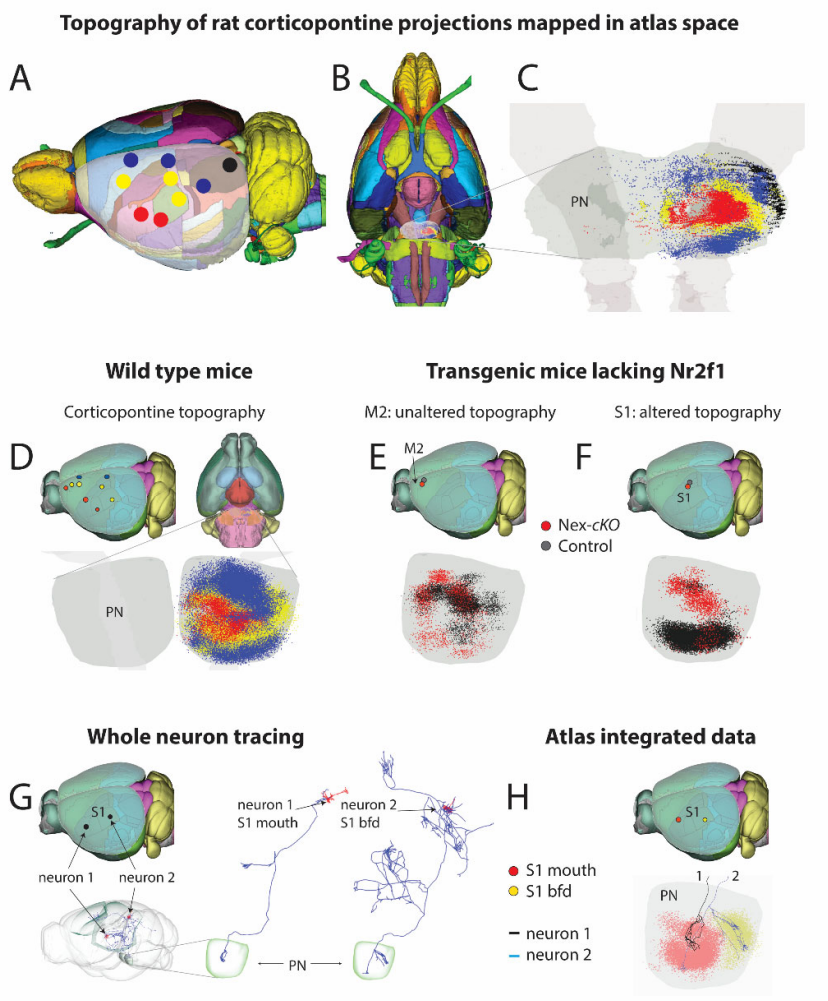

Fig. 4. Three-dimensional analysis of spatially integrated connections data

Examples illustrating studies of spatial organization in the first link of the rat and mouse cerebro-cerebellar circuit, based on tract-tracing data integrated and co-visualized in the Waxholm Space rat brain atlas (**A-C**) and the Allen Mouse Brain Common Coordinate Framework, CCFv3 (**E-G**). **A-C**, data combined from several rat brain experiments in which an axonal tracer was placed at separate locations in the cerebral cortex (**A**), and anterogradely labelled fibres were semiquantitatively represented as point coordinates spatially registered within the pontine nuclei. **B** shows the Waxholm Space rat brain atlas in view from ventral. **C** shows the outer boundaries of the pontine nuclei and descending corticofugal fibre tract as grey transparent surfaces, and data representing axonal labelling from different experiments as colour coded point clouds. Shifts in location of tracer injection sites from anterolateral to dorsomedial locations (**A)** corresponds to topographic shifts from internal to external lamellar subspaces in the pontine nuclei (**C**,). **D-F**, data

from a study of the impact of the cortical area-patterning gene *Nr2f1* on topographical organization of corticopontine projections in mice, combining tract-tracing data from the Allen Mouse Brain Connectivity Atlas with experimental data from transgenic mice lacking cortical expression of *Nr2f1* (40). **D**, co-visualization of color-coded 3D data points representing the distribution of corticopontine projections from different cortical locations in wild-type mice show a similar inside-out topographical organization as demonstrated in rats (50) (**A-C**). Comparison of the topographical distribution of pontine projections arising from corresponding locations in the secondary motor cortex (**E**), and primary somatosensory cortex (**F**) of Nex-*cKO* transgenic mice lacking *Nr2f1* and control animals demonstrates that corticopontine projections from the primary somatosensory cortex are altered in *Nex-cKO* mice, to resemble the projections from the secondary motor cortex. **G-H** illustrate data from different sources can be integrated and compared in atlas space. **G** shows whole brain reconstructions of two projections neurons (from the Janelia Research Campus MouseLight project, 21) located in the mouth and whisker representations of the primary somatosensory cortex, with a variable amount of profusely branching axons in several subcortical regions, including the pontine nuclei. **H** exemplifies a comparison of corticopontine projections from single neurons and anterogradely labelled projections from two populations of neurons in the mouth and whisker representations in the primary somatosensory cortex (data derived from the Allen Mouse Brain Connectivity Atlas, 5), indicating the trajectory of single cell projections relative to the point cloud representing the spatial distribution of projections from a larger amount of neurons in the same part of the cerebral cortex. The data shown in **A-C** were taken from (46, cases R113-BDA, R118-BDA, R124-BDA; 47, cases D55-FR; 48, cases M27-BDA, M27-FR; 49, cases R409-BDA, R412-BDA, R413-BDA). The data shown in **E**, **F**, and **H** were taken from (40; wild type cases: 100141780, 112229814, 112952510, 126908007, 127084296, 127866392, 141602484, 141603190, 585025284; Nex-cKO cases: 11643_17, 19423_7; littermate control case: 18035_1). The neuron reconstructions shown in **G** were taken from the Janelia MouseLight project (20, 21; https://www.janelia.org/project-team/mouselight; neuron AA0945, https://doi.org/10.25378/janelia.7804034 (#1), neuron AA1049; https://doi.org/10.25378/janelia.7822322 (#2)). Atlas surfaces and data points were co-visualized using the MeshView web application, RRID**:** SCR_017222, http://www.nitrc.org. Neuron reconstructions in **G** were visualized using the Scalable

Brain Atlas Composer (https://sba-dev.incf.org/composer; 51). Abbreviations: bfd, barrel field; M2, secondary motor cortex; S1, primary somatosensory cortex, PN, pontine nuclei.