Multimodal stereotaxic mouse brain atlas for robot-assisted, high-precision intracranial injection procedures in mice

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Background & Aim

Precision-targeting of distinct brain areas based on information from *in vivo* and *ex vivo* 3D imaging modalities holds great potential in preclinical target validation and drug discovery. We developed a multimodal mouse brain atlas based on brain templates from MRI, LSFM, and STPT including region delineations and skull-derived coordinate system to enable integration of findings across 3D imaging techniques and improved stereotaxic targeting.

Methods

Imaging

Male 10 weeks old C57Bl/6J mice were perfused with neutral buffered formalin. Mouse heads were imaged using X-ray micro-computed tomography (micro-CT) and T2-weighted structural MRI. The brains were dissected from skulls, iDISCO-processed, and imaged using LSFM.

Image processing

Standard landmarks were semi-automatically extracted from micro-CT skulls. Average MRI and LSFM brain templates were created using iterative registration and averaging algorithm. The templates were connected to the STPT-based Allen Institute's Mouse Common Coordinate Framework version 3 (AIBS CCFv3) via deformation fields. Skull landmarks were transferred to the MRI template, averaged, and used to create a stereotaxic coordinate system. The coordinate system and CCFv3 region delineations were transferred to template spaces of all modalities using deformation fields

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1 Workflow

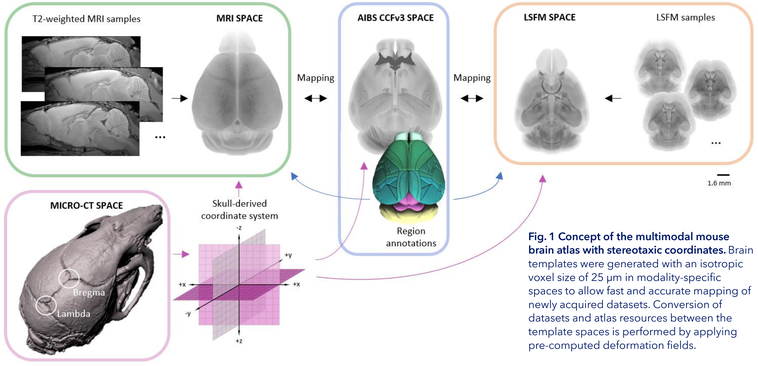

Fig. 1 Concept of the multimodal mouse brain atlas with stereotaxic coordinates. Brain templates were generated with an isotropic voxel size of 25 µm in modality-specific spaces to allow fast and accurate mapping of newly acquired datasets. Conversion of datasets and atlas resources between the template spaces is performed by applying pre-computed deformation fields.

2 Average skull landmarks

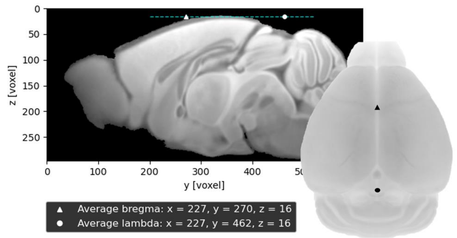

Fig. 2 Spatial positions of average bregma and lambda skull landmarks. Average bregma and lambda in sagittal and top view. The standard deviation for average bregma was 4.7 voxels in x, 5.8 voxels in y, and 1.9 voxels in z dimension. The standard deviation for average lambda was 3.3 voxels in x, 1.9 voxels in y, and 1.0 voxels in z dimension. Voxel has an isotropic size of 25 µm.

3 Stereotaxic coordinate system

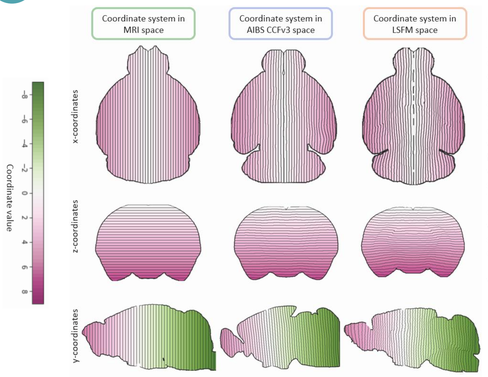

Fig. 3 Stereotaxic coordinates in MRI, CCFv3, and LSFM template spaces. The coordinate system was generated according to the convention used by Franklin and Paxinos in Mouse Brain in Stereotaxic Coordinates. The coordinate system is visualized in horizontal view for x-coordinates, coronal view for z-coordinates, and sagittal view for y-coordinates. The color scale indicates coordinate values for every voxel and equidistant (step size 250 µm from the origin) contour lines in black indicate levels at which the coordinate values are constant.

4 Web app for planning stereotaxic surgery

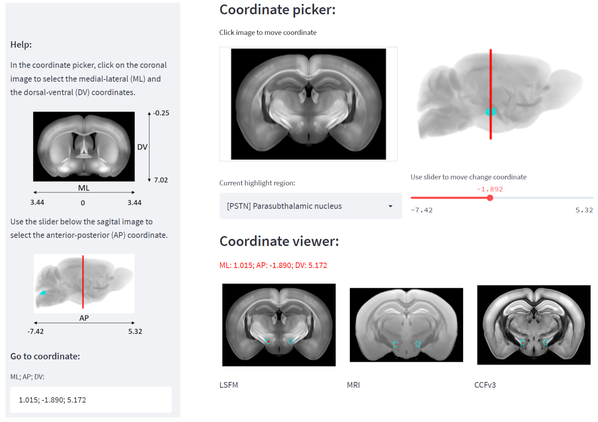

Fig. 4 Web interface for identification of *in vivo* coordinates. The app features stereotaxic coordinates in LSFM, MRI, and CCFv3 template spaces. The top panel is interactive – a sagittal view of a brain can be used to select a coronal slice and the coronal slice for pinpointing an anatomical structure. The lower panel depicts the position of the selected area in medial-lateral (ML), anterior-posterior (AP), and dorsal-ventral (DV) coordinates, and in three templates (red dot). It is also possible to navigate in the atlas by searching for a region using the dropdown menu (region highlighted in cyan) or by indicating a coordinate (left panel).

5 Robot-assisted stereotaxic injections based on atlas coordinates

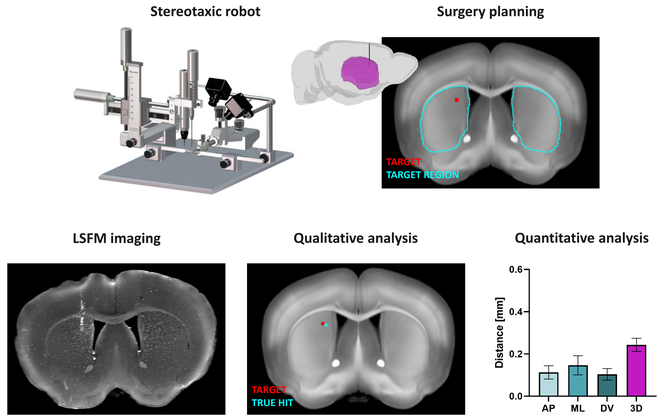

Fig. 5 Automatization of stereotaxic injections and evaluation of injection site accuracy using the multimodal atlas. A mock study was conducted to evaluate intracranial targeting accuracy using the Neurostar stereotaxic robot and the multimodal atlas. Light sheet microscopy of n=6 intracranially injected mouse brains was performed after tissue clearing to identify injection sites. Injection site segmentations were mapped to the multimodal atlas for qualitative and quantitative evaluation. Quantitative analysis of mouse brains injected into the dorsomedial striatum revealed that the distance between the target and true hit coordinates was on average 150 µm in the anterior-posterior (AP) axis, 110 µm in the medial-lateral (ML) axis, 100 µm in the dorsal-ventral (DV) axis, and 240 µm in 3D.

Conclusion

- Multimodal atlas allows the synthesis of complementary information from different imaging modalities and post hoc targeted manipulation of neural populations identified in image datasets
- CCFv3 region delineations and 3D coordinates enable accurate navigation in a mouse brain during stereotaxic surgeries
- Coupling of the atlas with a robotic stereotaxic system can improve the precision, consistency, and throughput of stereotaxic surgeries

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