Multimodal stereotaxic rat brain atlas for automated image analysis and data integration in 3D

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Background & Aim

Rats are often favoured in neuroscience research over mice due to their larger brain, more complex behaviour, and closer metabolic similarity to humans. However, acquiring high-quality and high-resolution datasets of molecular markers in intact rat brains is challenging. Advances in tissue clearing techniques and light sheet fluorescence microscopy (LSFM) now allow for detailed exploration of the entire rat central nervous system. The current project aims to adapt and extend the multimodal 3D stereotaxic mouse brain atlas pipeline developed by Perens [1] for the rat brain. This will enable streamlined analysis and facilitate the integration of datasets from various 3D rat brain imaging techniques.

Imaging Methods

Lean male rats 11-12 weeks old Sprague Dawley were perfused, and the skulls were deskinned and mounted for MRI and CT scanning using 130 and 30 µm isotropic voxel size respectively. Then, brains were dissected from skulls, processed with an adipoclear-based protocol, and imaged using LSFM at 10 µm isotropic voxel size.

References
[1] Perens et al. Neuroinformatics
https://doi.org/10.1007/s12021-023-09623-9

1 Mouse brain atlas workflow

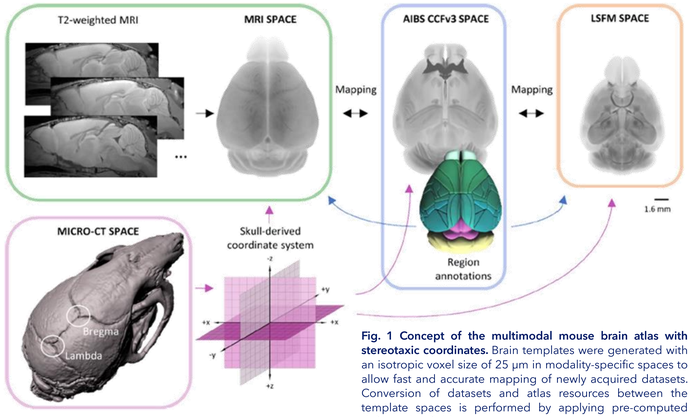

Fig. 1 Concept of the multimodal mouse brain atlas with stereotaxic coordinates. Brain templates were generated with an isotropic voxel size of 25 µm in modality-specific spaces to allow fast and accurate mapping of newly acquired datasets. Conversion of datasets and atlas resources between the template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

2 Adapted pipeline for rats

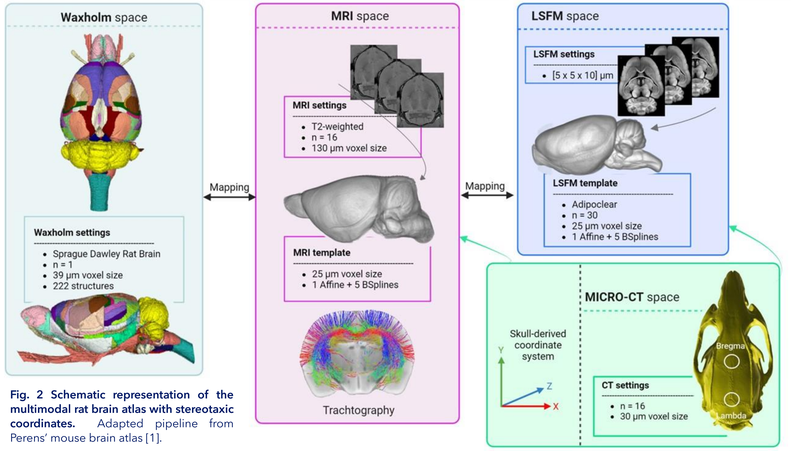

Fig. 2 Schematic representation of the multimodal rat brain atlas with stereotaxic coordinates. Adapted pipeline from Perens' mouse brain atlas [1].

3 LSFM template

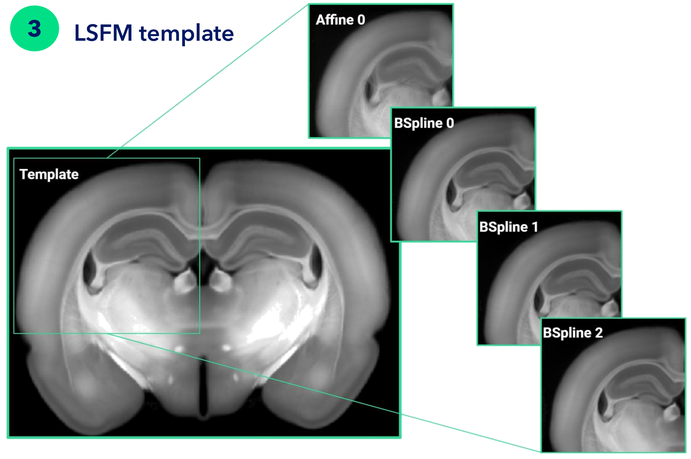

Fig. 3 LSFM Template. Iterative registration process of LSFM samples to generate a LSFM template. Registration consists of 1 Affine DTI registration followed by 4 BSplines. All the registration process has been performed in using ITK Elastix in Python.

4 TH staining in a rat brain

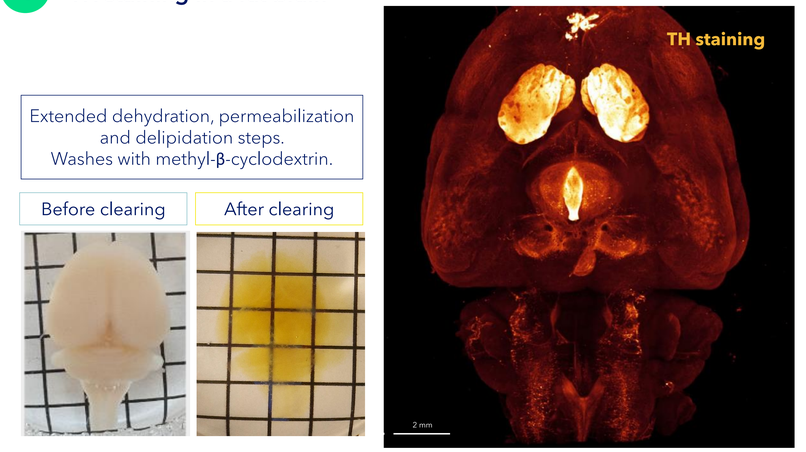

Fig. 4 Example of implementing tissue clearing and light sheet microscopy in the rat brain. Extended delipidation with detergents and methyl-β-cyclodextrin assist in rat brain LSFM. Rat brain before and after clearing, and after 3D light sheet image acquisition of tyrosine hydroxylase (TH) staining.

Imaging Processing

Following image acquisition and postprocessing, a 3D spatial MRI and LSFM template are generated as a population iterative registration (1 Affine + 4 Bsplines) of 16 and 30 rat brains respectively. Templates are constructed with 25 µm isotropic voxel size. Labels are then transferred with whole-brain one-to-one registration from the Waxholm atlas to the MRI template and subsequently to the LSFM template with multi-regional registration. In adittion, CT brains are registered one-to-one with their MRI skull. Bregma and lambda are detected from every skull and then averaged to create a population-average stereotactic coordinate system.

What do we aim next

Next step is to visualize gene expression, connectivity or neural activation in a whole brain at single cell level. The 3D information from the imaged brains can be registered to a reference atlas. Once aligned, the resulting data can be compared or overlayed with other maps created using the same atlas.

Conclusion

This atlas framework bridges the gap between in vivo and ex vivo rat brain imaging and facilitates data collection, sharing, and comparison across multiple experiments within the same space.

+ It enables the utilization of signal contrasts from multiple modalities and markers to enhance the precision of region delineations of the atlas.
+ It ensures precision during stereotaxic surgeries.
+ It is designed to accommodate the inclusion of additional brain templates based on different signal-generation mechanisms.

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