Whole-brain three-dimensional imaging for quantification of drug targets and treatment effects in mouse models of neurodegenerative diseases

Quantitative histopathology in preclinical neuroscience: Altered brain functionality in neurodegenerative diseases, including Parkinson's disease (PD) and Alzheimer's disease (AD), involve complex pathological changes and molecular mechanisms which poses a major challenge for development of effective drug treatments. Progressive loss of specific neuron types and projections are hallmarks of PD and AD and proper histological evaluation of relevant disease models therefore requires highly reliable cell detection and quantification methods. Immunohistochemistry is the prevailing method of choice to visualize and quantify histopathological changes in brain tissue sections. Although stereology applied to serially sampled sections is considered the gold standard for unbiased three-dimensional (3D) estimation of histomorphometric changes and therapeutic effects in neurodegenerative models, the elaborate sequence of histological processing steps can be time-intensive and limit analyses to a few preselected brain areas. Whereas physical tissue sections may provide higher structural resolution for any given 2D plane, the advantage of volume imaging is to facilitate 3D investigation of brain structures and cell populations in the intact brain. In this respect, clinical whole-brain 3D imaging has continued to provide valuable insights in early detection, diagnosis and interpretation of PD and AD in humans, yet it has remained challenging to establish in preclinical research and drug discovery.

Light sheet fluorescence microscopy (LSFM) has emerged as a powerful tool for unbiased mouse whole-brain 3D image analysis, offering unique advantages for a diverse range of applications in preclinical neuroscience. In contrast to mechanical tissue sectioning, LSFM permits 3D imaging at micrometer resolution which provides unbiased means to study the distribution of labeled neurons, axonal projections at a whole-brain scale. Considerable progress has been made to improve methods for organ clearing and immunolabeling, each offering advantages in tissue processing and LSFM visualization of fluorescent proteins, transgenic reporters or specific antigens (Ueda et al., 2020). The low brain anatomical variation between individual mice allows LSFM scanned brains to be mapped onto a common 3D volumetric brain atlas, for example the Allen Mouse Brain Common Coordinate Framework (CCF), which can be used to computationally map the identified fluorescent signal to specific brain regions (Fürth et al., 2018).

We have in a recent publication highlighted the applicability of LSFM for high-throughput, brain-wide quantification of histopathological hallmarks in a standard mouse model of PD (Roostalu et al., 2019). In this perspective article, we will emphasize the role of LSFM for whole-brain 3D mapping and quantification of drug targets, drug distribution and therapeutic effects in mouse models of PD and AD.

Quantitative LSFM in preclinical drug discovery: We will here highlight key elements of LSFM and 3D image analysis which should be considered when applying LSFM in preclinical CNS drug discovery. For a more detailed discussion on technical aspects, we refer to more comprehensive reviews of the subject (Mano et al., 2018; Ueda et al., 2020). LSFM has been instrumental for 3D reconstruction and mapping the complex architecture of specific neuronal populations and long-range axon tracks which lays the foundation for generation of mouse whole-cell atlases and detailed circuitry maps (Renier et al., 2016; Fürth et al., 2018; Mano et al., 2018). Although technological advances within LSFM continue to provide detailed insight into CNS cell topography and connectivity, high-resolution whole-brain imaging is generally not well-suited for quantitative histology due to slow acquisition speed and considerable computational power required for extraction and interpretation of large-scale data sets. To overcome these challenges there is a need for implementation of procedures which can increase sample throughput and streamline large-scale LSFM data management.

Currently, scanning time represents a critical rate-limiting factor in quantitative LSFM studies. Depending on the level of detail, scanning may take up to 24 hours to acquire whole-brain image stacks at high resolution. However, despite the impressive amount of information this approach is not compatible with study designs that include a relatively high number of samples to ensure proper statistical power. Various LSFM strategies can be used to determine CNS compound effects at different levels of anatomical resolution. For example, lower spatial resolution is advantageous for initial screen of brain-wide changes in fluorescent signal intensities as a proxy for target expression. This approach preserves high acquisition speed and can qualify selection of brain areas of specific interest for further detailed analysis. By re-scanning selected areas at higher magnification, labeled single cells can be visualized and segmented out for unbiased 3D counting (Roostalu et al., 2019). For validation purposes, LSFM fluorescent signals in specific brain regions are usually compared to immunolabeled tissue sections sampled from the contralateral hemisphere or separate brains (Renier et al., 2016; Liebmann et al., 2016). Alternatively, cleared organs (iDISCO) together with LSFM and deep-learning assisted data analysis has proven highly efficient for automated detection, anatomical mapping and quantification of LSFM-imaged molecular targets in whole organ mounts. LSFM-deep learning is particularly applicable for whole-brain classification and detection of CNS cell phenotypes (Fürth et al., 2018) and probe signal intensities (Renier et al., 2016; Salinas et al., 2018). Once established through interdisciplinary collaboration between biologists and bioinformaticians, LSFM-deep learning platforms enable for unbiased whole-brain histomorphometric analysis of large number of samples (Roostalu et al., 2019).

LSFM imaging in models of PD: In preclinical PD research, animal model development has centered around recapitulating progressive dopaminergic neuronal loss in the nigrostriatal system which is fundamental for development of the debilitating cardinal motor deficits in PD. In animal models of PD, estimation of dopaminergic neuron numbers and projections is therefore imperative for model phenotyping and assessment of drug treatment effects. In this context, immunohistochemical detection of cells positive for tyrosine hydroxylase (TH) expression is an essential tool for visualization and quantification of catecholaminergic cells, including dopaminergic neurons. Current immunohistochemical assessment of midbrain TH-positive neurons and projections in standard mouse models of PD have yielded highly variable results which is likely explained by the various histomorphometric methods applied. In addition, conventional immunohistochemical techniques have yielded insufficient spatial information on TH-expressing neurons in the mouse brain, making it unclear to what degree dopaminergic cell architecture in mouse models of PD compares to the human condition. Consequently, there is a need for improved methods that allow for more complete, accurate morphological and quantitative characterization of dopaminergic neurons and projection pathways in animal models of PD.

To create an unbiased and comprehensive map of TH-positive neurons and projection pathways in the mouse brain, we developed a method combining immunolabeling-enabled 3D imaging of solvent cleared organs (iDISCO) together with LSFM and deep learning analysis. This pipeline enables high-throughput automated 3D mapping and quantification of TH signal intensities throughout the intact mouse brain (Figure 1A). Fluorescence signals were further assigned to dopaminergic and noradrenergic regions by superimposing the whole-brain TH reference map with CCF-registered gene expression markers from the Allen Brain Atlas. The iDISCO-LSFM platform was extended to perform unbiased counting of...
the total number of midbrain TH-positive neurons. When applied to the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse, one of the most common dopaminergic neurotoxin models applied in preclinical PD research, we confirmed substantial loss of TH-positive neurons in the substantia nigra and ventral tegmental area with numerical changes in dopaminergic neuron numbers that lies well within the ranges reported using stereology. In addition, altered TH expression patterns were detected in brain structures not previously associated with MPTP neurotoxicity. The collateral changes included reduced TH expression in several other basal ganglia-associated nuclei paralleled by increased TH expression in various nuclear subdivisions of the amygdala and hypothalamus. Collectively, the study underscores that regulation of central TH expression in the MPTP mouse is more widespread and dynamic than anticipated from previous conventional histological studies. Improved understanding of neurodegenerative and compensatory molecular mechanisms is fundamental for promoting effective treatments for PD. Our LSFM study therefore sets a framework for defining the underlying mechanisms for MPTP-induced TH adaptive changes in the limbic system, which may possibly associate to abnormal amygdala and hypothalamic activity implicated in the emotional and endocrine disturbances in PD. In addition to histochemical validation of PD models, LSFM deep learning pipelines are advantageous in providing fast and highly resolution quantitative assessment of whole-brain TH responses to compounds in preclinical development for PD. To provide further information on molecular signaling, our 3D whole-brain reference map of TH expression can be superimposed with other LSFM-imaged and CCF-registered molecular targets, e.g. cell activation markers (Salinas et al., 2018), for better interpreting signaling mechanisms involved in drug therapeutic effects. The potential of LSFM-based 3D quantitative TH analysis generalizes to histological studies in preclinical models of diseases characterized by deficient catecholaminergic neurotransmission also including schizophrenia, drug abuse and obesity.

Another important aspect of LSFM is the ability to directly image and quantify biodistribution of therapeutic peptides and antibodies following systemic administration (Salinas et al., 2018). In the context of AD, performing imaging of specific molecular targets, e.g. hyperphosphorylated tau epitopes and markers of gliosis, multitemporal quantitative LSFM analysis can further inform about the regulation of disease-relevant targets in mouse models of AD.

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LSFM imaging in models of AD: AD is characterized by slow but progressive cognitive decline with a defining neuropathology of abnormally high densities of extracellular amyloid β (Aβ) senile plaques and intraneuronal accumulation of neurofibrillary tangles (composed of a hyperphosphorylated, aggregated form of microtubule-binding tau protein) in combination with neuronal loss and brain atrophy. Targeting plaques and neurofibrillary tangles therefore remain a rational approach for treatment of AD, however, the relationship between the deposition of these neurodegenerative markers and the onset of AD remain largely unknown. Various AD mouse models have been engineered to overexpress mutant forms of human amyloid precursor protein (APP) and tau which promotes production of Aβ and hyperphosphorylated tau. Many of these models display disseminating Aβ plaques and/or neurofibrillary tangles which develop in an increasingly complex brain regional patterns with age. By providing more complete information on the spatiotemporal and quantitative changes in histopathological hallmarks, LSFM can further qualify murine AD models with respect to human translatability and utility in preclinical drug discovery. In accordance, LSFM has emerged as an advantageous method for unbiased mapping and quantification of β-amyloid plaques and hyperphosphorylated tau protein in mouse models of AD (Liebmann et al., 2016; Detrez et al., 2019; Gail Canter et al., 2019). Unlike APP models developing memory loss and Aβ plaques with little or no neuron loss, tau-overexpressing mouse lines are often characterized by more severe neuropathology. This is particularly evident in mice expressing a regulatable mutant tau transgene (rTg4510) which is strongly associated with development of frontotemporal dementia. Early accumulation of pathological tau species in this standard mouse model of tauopathy coincides with significant depletion of forebrain neurons and synaptic markers (Helboe et al., 2017). To obtain further information of the brain morphological changes in this model, we performed LSFM-deep learning quantitative volume analysis across the entire brain of rTg4510 mice. Following reference atlas-guided segmentation of annotated brain regions, the precise volume of each region of interest was calculated. Compared to wild-type mice, discernible volume loss was observed within several brain structures of rTg4510 mice, most extensively in the cortical mantle (Figure 1B). The development of cerebral atrophy in rTg4510 mice is consistent with gross brain volume changes determined by structural magnetic resonance imaging (Holtman et al., 2016). Hence, volumetric LSFM provides a sensitive measure of brain atrophy. When coupled with immunolabelling for e.g., hyperphosphorylated tau epitopes and markers of gliosis, multi-parametric quantitative LSFM analysis can further inform about the regulation of disease-relevant targets in mouse models of AD.

Conclusion: LSFM applied to cleared whole-brain samples can overcome the inherent variability and technical limitations associated with employment of traditional 2D-based histomorphometric techniques. Implementation of high-throughput LSFM analysis coupled with deep learning computational methods for fast processing of big data represents an ideal technology for absolute quantification of histopathological hallmarks, potential therapeutic molecular targets and drug distribution in preclinical models of neurodegenerative diseases. By combining information on quantitative molecular changes and drug distribution at single cell resolution, quantitative LSFM provides an important tool for better interpreting therapeutic drug effects in mouse models of CNS diseases. In particular, LSFM-deep learning platforms have proven efficient for unbiased whole-brain detection and quantification of histopathological hallmarks in mouse models of PD and AD, which paves the way for routine use of quantitative LSFM in the assessment of therapeutic effects of test compounds in preclinical development for these neurodegenerative diseases.

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were compared in control mice (purple) versus coordinated framework, CCF) average fluorescence signal intensities (A) Following registration into the Allen Mouse Brain atlas (common Parkinson’s disease (PD) and Alzheimer’s disease (AD). quantitative whole-brain 3D imaging analysis in mouse models of Figure 1 Light sheet fluorescence microscopy (LSFM) for visualize differences between study groups. PD model: Changes in tyrosine hydroxylase (TH) expression and total counting of TH-expressing

Aducanumab-like antibody following intravenous administration in transgenic APPPS1-21 mice (13 months of age). Aducanumab-like antibody displays significant cortical atrophy at 6 months of age.

References


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