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Multiplexing High-Resolution Whole Organ Analyses of Mammalian Tissue
Fach/Einrichtung: Physiologie
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I established imaging and analysis modalities that enable image-based sub-micron resolution quantification of large whole-organ samples to address questions in systems biology, axonal wiring, neuroanatomy, and PD using high throughput/high content ISH/IHC protocols, a variety of state of the art microscope modalities, and automated, 3D analysis methods that I setup or developed specifically to address these challenging questions.

Setting up a novel method for determining, visualizing, and quantifying expression patterns of ORs in the OE using serial sections of mouse crosses containing GFP/RFP tagged ORs gave a first glimpse at the true appearance of these patterns and established the protocols necessary to evaluate them using the latest methods available. The 3D OR pattern evaluation paradigm was then improved by setting up a robotic system for high-throughput ISH enabling systematic staining using ISH probes generated against a large sample of OR genes. I established a new three-color multiplexed regimen that permitted analysis of up to 15 different ORs in the same mouse and validated the method before establishing a new toolkit of quantitative methods geared at analyzing and comparing these patterns. I used a variety of analytical measures to describe the distribution of ORs within their zone and performed exploratory data analysis to determine zone grouping that was then tested for statistical significance. Using these tools I mapped the expression patterns of 70 ORs with sufficient precision to group them into zones, some of which like 256-17, 109-1, and 33-1 break the rules of all existing OR expression models. I followed up on the zonal groupings with a bioinformatics analysis and identified candidate TFBS targets for further experiments. I then focused on one family of candidate TFFs with an ISH-based expression analysis.

In the bulb, I used ΔOR mice to analyze projection patterns and collected preliminary data that indicates the glomeruli belonging to second-choice ORs create subdomains in the bulb. I followed up with initial experiments suggesting these domains are OE zone-locked implicating OSN-type based OR-choice bias. I then setup a new imaging modality to investigate OSN axon projection patterns in the bulb and made measurements of the positions of 352 glomeruli. I then quantified the positional variability for 11 glomerular domains belonging to 6 ORs. I found that inter-individual variability among age-matched mice matches that of intra-individual variability and that the degree of variability is OR-dependent. I showed this variability is stable with age, as is the variability in number of OR-defined glomeruli per half-bulb. My results quantify the level of precision that is delivered by the mechanisms of OSN axon wiring, differentially for the various OSN populations expressing distinct OR genes.

The methods work at higher resolution as shown in the project using sub-cellular SHG analysis and measurements on YMs in muscle fibers. The methods were also used on large human olfactory bulb samples in which I mapped the distribution of glomeruli throughout the bulb to provide an anatomical view of the glomerular organization and how it compares with that of a mouse. I analyzed 11 bulbs, six control and five PD and profiled the distribution of glomeruli and showed that total glomerular volume is lower in PD cases versus non-PD in a statistically significant manner. These findings highlight the importance of studying whole-organ samples with sub-micron resolution to provide clues to answering crucial questions related to neuro-anatomy/genetics and how that relates to diseases such as Parkinson’s.