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Architecture of a glomerular domain revealed by a novel volumetric electroporation technique

Fach/Einrichtung: Anatomie und Zellbiologie

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In my thesis, I addressed two complementary, yet separate issues: The technical development of a methodological approach capable of delineating medium-sized neuronal networks and in a second step its application to the circuit of an identified olfactory glomerulus. This provided the basis for subsequent, quantitative morphological network analysis.

The results presented in the first part of this thesis constitute a detailed and systematic characterization of conventional and FIB-modified glass microelectrodes for targeted electroporation. By combining *in silico* modelling and *in vitro* as well as *in vivo* experiments I have shown that nanotechnological modification of conventional glass micropipettes can be used to enhance the applicability and efficiency of targeted electroporation.

In the second part of my thesis, the first comprehensive dataset of the associated circuitry of an identified olfactory glomerulus is presented, comprising absolute numbers of different cell types, spatial distribution patterns and fine-scale morphological descriptions. This allows an insight into the structural architecture of the processing module of the MOR174-9 glomerulus and reveals the quantitative framework for possible future studies involving dense circuit reconstruction techniques.

Based on the comprehensive morphological dataset shown here, a unified and simplified nomenclature of glomerulus-affiliated projection neurons can be proposed based on morphological parameters subdividing the projection neurons into external TCs (eTCs), superficial TCs (sTCs), middle TCs (mTCs), deep TCs (dTCs) and MCs.

I have shown that the main projection neurons, both MCs and dTCs, are not distributed randomly across the MCL but form a cluster which is preserved across animals. The detailed analysis of the local lateral dendritic projectome of the projection neurons shows a sharp spatial segregation along the 'vertical', i.e. deep-to-superficial, axis and also reveals notable differences in the 'horizontal' distribution pattern. These observations suggest distinct functional roles of the various classes of projection neurons which may be involved in separate signal processing subsystems.

Most surprising, however, has been the detection of a highly specific association between principal projection neurons – especially anatomically identified MCs – and a dense axonal hotspot in the adjacent IPL. This provides anatomical evidence of a putative feedforward TC→GC→MC connection within a given glomerular module since MCs themselves were not found, and are highly unlikely, to be the source of such local axon collaterals.

Taken together, I have devised and described an approach for the functional dissection of the glomerulus-associated circuitry of the genetically-tagged MOR174-9 glomerulus. By means of nanoengineering, the established technique of targeted glass micropipette electroporation could be adapted so that a volumetrically exhaustive electroporation and sparse feature detection have become feasible. This has provided new insights into the building architecture of the glomerular domain. Moreover, these advancements may render the novel technique a promising method for future projects involving larger or geometrically more difficult structures.