Chapter 4 - Summary and outlook

This thesis aimed to investigate whether there exist a positive structure-function interrelation between the specific arborization pattern of a dendritic tree and the changing function of an identified central neuron. The intriguing process of insect metamorphosis sets the frame of the work. A prominent feature of metamorphosis is the structural remodeling of neuronal architecture, which here will be exemplified through a well documented model system - the dendritic tree of the identified motoneuron 5 (MN5). The MN5 is part of the flight motor-network of the holometabolous insect *Mandca sexta*. Its structural dynamics offer the opportunity to address fundamental questions that recently took center in developmental neurobiology, such as: Does the dendritic structure of a neuron hold a share on the functional output by its architecture? What are the underlying mechanisms determining the highly coordinated shaping of dendritic structure?

Both manuscripts make use of novel 3-dimensional geometric reconstruction tools from confocal images stacks, sampled at the limit of optical resolution imposed by light microscopy. Hereby it was possible to quantitatively asses the distribution of labeled proteins along the neuronal surface, in particular the synapse distribution throughout an entire dendritc tree.

In the 1st manuscript (Chapter 2) I present experiments which were designed to examine the dendritic structure and synapse distribution of the MN5 during development, focusing on the comparison of larval and adult morphology. I could show that GABAergic synapses are placed on the dendritic arbor, following stage specific distribution rules which clearly demonstrate a sub-dendritic targeting of synapse placement. In addition, multi-compartment simulations of the assessed sub-dendritic synapse distribution rules using solely passive models have been performed. These models provide a strong indication that in a purely passive dendritic tree, the stage specific branch pattern and sub-dendritic synapse distribution can contribute to a behaviorally adequate firing output. These computational experiments were not intended to include realistic properties of the neuron, but could demonstrate that both structure and synapse placement can work in cooperation to support the generation of adequate behavior, only using the passive integration properties of the dendrite. It will be interesting to test how these predictions will hold when multi-compartment models of MN5 are modified to include more realistic attributes like active ionic conductances

which have already been shown to exist in the dendrites of MN5. Further, it would be interesting to investigate other transmitter classes in the future, but also to label the postsynaptic site, the receptors. This would require either the production of antibodies, or one could use GFP tagged receptors in *Drosophila*. Finally, it will be important to use motoneurons with fewer dendrites for this analysis in the future, because reconstruction of MN5 with approximately 9.000 dendrites and a total length of approximately 40.000 µm is very time consuming, even with the novel methods applied in this study.

The second manuscript addresses effects of the pharmacological blockade of chloride currents on postembryonic developmental processes by systemic injections of picrotoxin. Conditions of hyper-excitability within the nervous system during metamorphosis increased the branching rate, resulting in an overgrowth of the MN5s' dendritic tree, a decreased density of GABAergic synapses and affected motor performance of the adult. These results strongly indicate impairment of dendritic growth and synaptogenesis caused by a missing chloride influx. However, blocking GABAergic transmission by systemic injection remains speculative since the spectrum and mechanisms by which systemic PTX injections mediate the observed alterations is unknown.

According to the results presented here I also propose several future experiments that might offer promise towards a greater understanding of the interplay between architectural features and behavioral demands: First, distinct picrotoxin induced structural changes of dendritic architecture have been interpreted as a possible structural homoeostasis mechanism. One could control this directly by applying the same compartmental-model simulations testing for an effect on this change in structure. This could extend the dimension of homeostatic capability or mechanisms for compensation of proper neuronal function. Second, it is of great interest to find presynaptic neurons to the MN5. One could correlate the measurements of electrophysiological recordings on intrinsic membrane properties, spiking capability and integration of activity into network function to precise measurements of 3-dimensional reconstructions and evaluate presented results on GABA synapse distribution patterns or other transmitter molecules on the computational power of the MN5. Finally, the model system of the MN5 in the fruit fly *Drosophila melanogaster* could be regarded as an attractive tool to elucidate further findings in the intriguing process of dendritic development and computation. Modern genetic tools and genetic manipulation opportunities applied to

Drosophila allow to systematically screening for the contribution of certain aspects on developmental neuronal wiring processes and behavioral consequences. Targeted manipulation in individual neurons would help to dissect apart possible mechanisms by which PTX might act. Targeted manipulation by knock-out experiments or over-expression of GABA receptors within the *Drosophila* motoneurons for example would represent an elegant way to either investigate a state of hyper- or hypo-excitability of the motoneurons.