

## Chapter 1 - Introduction

Neuronal development constitutes the fascinating process of assembling, as it seems, an almost infinite number of neurons in appropriate circuits that are essential for adequate brain function. Interconnections between neurons allow transferring electrical activity from one relay point to the next. This electrical activity is shaped and filtered through many different processes within a neuron and we just start to understand how they work together to ultimately initiate a recognizable and adequate behavior. The discovery of Ramon y Cajal that these brain cells seem to be of a polarized nature, hypothesizing that they receive information at the one end (dendrites) and relaying it to the other (axons) was probably the initial discovery which laid the basis for modern cellular neuroscience (the neurone doctrine; Cajal, 1897; Sotelo, 2002). He further noticed that neurons can be categorized into different anatomical classes which are repeatedly observable between animals. Indeed, this anatomical discrimination of different classes of neurons, primarily based on their dendritic morphology, is also reflected in different electrophysiological and computational differences (Agmon-Snir et al., 1998; Haag et al., 1997, 1999, Borst and Haag, 1996). However, examination of the neuron doctrine indicates that it no longer encompasses all important aspects of neuron function (Bullock et al., 2005). For example, intracellular recordings from dendrites of cerebellar purkinje cells and hippocampal neurons proved that dendritically initiated action potentials could influence the signal propagation within dendritic trees and their firing ability (Llinjás and Nicholson, 1971; Golding and Spruston, 1998; Liu, 2004; Wong et al., 1979; Johnston et al., 1996). Back propagating action potentials into dendrites influences their computational capability (Stuart et al., 1993). The functional complexity of dendrites and the role they play in synaptic integration and plasticity objects a simple receiver function of dendrites (Bullock et al., 2005). Therefore, the role of dendrites for the neuronal output of a cell may be far more complex as the primary neuron doctrine implies.

The shape and size of dendrites of different classes of neurons is very diverse, ranging from only a few branches to trees with hundreds of branch points and segments. The stereotypic repetition of the pattern of growth between individual animals but the same neuronal class is common to all animals with a centralized nervous system. In vertebrates, pyramidal and purkinje cells each represents a very prominent group of cell highly abundant neuron classes in the higher processing centers of the vertebrate brain.

Yet, invertebrates with much smaller brains commonly have fewer “copies” of neurons belonging to the same class and even offer the possibility to readily identify individual neurons, which are unique in form and their network function (identified neuron concept). Well known examples are the abdominal giant interneurons of crickets and cockroaches (Mendenhall and Murphy, 1974; Mizrahi et al., 2000), giant interneurons of the vertical system in dipterans, like i.g. in *Drosophila* lobula plate of the optic lobe (Bushbeck and Strausfeld, 1997; Scott et al., 2002) or an even more dramatic example of morphological invariance between neurons of the same type are insect motoneurons, for example in the holometabolous insect *Manduca sexta* (Consoulas et al., 2000; Duch and Levine, 2000, 2002; Weeks and Levine, 1990). In some ideal cases, in invertebrates well defined behavioral functions can sometimes be related to the activity of a single identified neuron, despite the fact that this neuron is still part of a network (Hammer, 1993).

Shape and complexity of dendrites are hallmarks of neuronal identity, and although the morphological similarity between neurons of the same class is obvious, there is still variability in the branching topology further distant to the root of the dendritic tree (Libersat and Duch, 2004; Libersat, 2005). The morphological homogeneity within a neuronal group can be interpreted as a hint that neuronal architecture is related to function of a given neuron. And indeed, dendritic morphology crucially shapes the integration of synaptic input and influences the dynamics of firing action potentials. (Connors and Regehr, 1996; Häusser et al., 2000; Vetter et al., 2001; London and Häusser, 2005). Therefore, it seems only logical that the spatial pattern of the dendritic tree might be important for its function. This however is a conclusion which is difficult to prove, as the function of a nerve cell can only be described if their involvement into a specified behavioral task is known. This seems difficult, but not impossible to prove with neurons of higher brain centers in vertebrates (Nolan, 2004), but even more achievable on identified neurons with a specified behavioral output.

The majorities of excitatory and inhibitory acting synapses terminate on dendrites. Their delicate interplay by summation of EPSPs and IPSPs determines the computational output of a given neuron in dependence to both, active and passive properties of a given dendritic tree. The structural arrangement and functional balance of excitatory and inhibitory inputs within a dendritic tree are thought to be organized in a constant ratio to ensure meaningful interaction within dendritic trees (Liu, 2004). With respect to targeted synaptic input patterns within dendritic trees little is known about the

functional consequence, or the impact on dendritic computational processes. Few studies demonstrate targeting of synaptic inputs as for example in mice neocortex, where different types of GABAergic inputs are directed to the perisomatic and the distal dendritic regions of pyramidal neurons (Somogyi et al., 1998; Di Cristo et al., 2004). Similarly, with regard to excitatory inputs the number of AMPA-type glutamate receptors is increased at distal dendrites of pyramidal cells (Stricker et al., 1996; Magee and Cook, 2000; Andrasfalvy and Magee, 2001). Such findings suggest rules for targeted synaptic input patterns within dendritic trees. Since systematic organization of synaptic contacts is a far more complex and cost dependent task than a random interconnection, a functional role seems to stand the reason. Attracted by these thoughts the question arose, even in the microscopic dimensions of neurons which integrate, filter and amplify electric discharges, whether their form and synaptic input patterns are optimized for their specific function, i.e. to support in the most favorable way, the generation of adequate behavior?

### **1.1 *Manduca sexta* and the identified motoneuron 5 during postembryonic development as model system**

Insect metamorphosis represents a model to study developmental changes that allow an adult insect to perform a vastly different suite of behavioral performance as compared to larval stages. The acquisition of the behavioral alterations requires modifications within the nervous system and its circuitry as well as changing synaptic connectivity (Consuolas et al., 2000; Tissot and Stocker, 2000, Landgraf and Thor, 2006). The larval behavior of *Manduca sexta* includes crawling, feeding, defensive thrashing and ecdysis, whereas the adult insects walk, fly and reproduce (Consuolas et al., 2000). The identified motoneuron 5 (MN5) of the holometabolous insect *Manduca sexta* participates in the larval stage in crawling behavior and it innervates a larval dorsal body wall muscles. After metamorphosis, in the adult, it participates in flight behavior by innervating the dorsal longitudinal flight muscle (Duch et al., 2000). The MN5 is a well described identifiable model neuron for detailed description of metamorphic changes in relation to its dendritic morphology, intrinsic membrane properties and conductivity (Duch and Levine, 2000, 2002; Libersat and Duch, 2002), its synaptic inputs (Duch and Mentel, 2004; Evers et al., 2006) and activity and calcium-dependent (Duch and Mentel, 2003; Burkert and Duch, 2006) as well as hormonal dependent developmental processes (Weeks and Truman, 1986; Weeks and Levine, 1990; Levine

and Weeks, 1996; Weeks, 2003; Börner et al., 2006). The remodeling during metamorphosis starts with retraction of larval arborizations and dismantling of synaptic connectivity, reaching a maximum of dendritic retraction at the beginning of pupal life, followed by an early phase of massive dendritic growth and a phase of dendritic refinement in late pupal life (Duch and Levine, 2000; Libersat and Duch, 2002). In the adult dendritic structure of a neuron and its synaptic connections are the prerequisite that substantiates a correct integration within its neuronal circuitry. Beyond a different ion channel facility after metamorphosis, this structural remodeling of the dendritic tree of MN5 is thought to have important impact on fulfilling adult behavioral requirements. However, this metamorphic transition is a well suited model for the investigation of postembryonic developmental alterations in dendritic structure and offers the opportunity to relate it to function.

## **1.2 Goal of the thesis**

In this thesis I aimed to analyze the interplay of “form and function” of the MN5 of *Manduca sexta* by investigating the variability of dendritic structure in dependence to different developmental stages (chapter 2) to find further arguments that stress the relation of dendritic morphology to function. Are there rules for sub-cellular targeting of synaptic inputs within dendritic trees that influence the computational output of a given neuron? I considered the neurotransmitter  $\gamma$ -amino butyric acid (GABA) within the dendritic tree of MN5 as particularly interesting for two reasons: first, the balance of excitatory and inhibitory inputs within a dendritic tree is thought to ensure meaningful interaction within dendritic trees (Cline, 2005; Liu, 2004). Second, to ensure adequate contractions of the DLM flight muscle the activation of MN5 must occur at the correct time point within each wing-beat cycle. The hypothesis was that the necessary temporal precision of the MN5 within flight rhythm is correlated to the inhibitory GABAergic input, since different studies demonstrated that GABAergic interneurons projecting on flight motoneurons do contribute on flight performance (Judge and Leich, 1999; Richardson and Leich, 2007). How variable is neuronal morphology for neurons of the same type? What is the resulting difference of dendritic geometry to the computational output? As a prerequisite for a qualitative neuronal modeling and tackling complex questions like these, a quantitative microanatomical description of dendritic architecture is needed. For this purpose previously developed precise 3-dimensional dendritic surface reconstruction tools (Schmitt et al., 2004), automated co-localization analysis to

map the distribution of potential input synapses through entire dendritic trees (Evers et al., 2005) and compartmental modeling of the stage specific geometric reconstructions were performed.

What are the developmental factors that shape mature dendritic structure apart from the so far known genetic factors (Mizrahi et al., 2000; Scott et al., 2002; 2003), hormonal cues (Weeks and Levine, 1990; Levine and Weeks, 1996; Consoulas et al., 2000; Weeks, 2003) and activity-dependent developmental processes (Hua and Smith, 2004; Libersat and Duch, 2004)? In a second approach (Chapter 3) the manipulation of neuronal excitability during postembryonic development through injection of the non-competitive GABA<sub>A</sub> receptor antagonist picrotoxin were performed. I aimed to investigate a possible developmental role of GABA induced inhibition via chloride influx on dendritic architecture. Picrotoxin is known to induce hyper-excitability within the nervous systems (Stilwell et al., 2006). In different systems, multiple roles for GABA<sub>A</sub> receptor activated chloride channels have been reported during different phases of development (Ben-Ari, 2002; Akerman and Cline, 2007), but the contribution to developmental processes in insects remains unclear.

The results are presented in two chapters based on manuscripts ready for submission:

Chapter 2:

Meseke M, Evers JF, Duch C: Sub-dendritic synapse targeting and postembryonic dendritic geometry remodeling of an identified neuron subserve its changing behavioral role

Chapter 3:

Meseke M, Duch C: Dendritic remodeling but not sub-dendritic GABAergic synapse targeting is affected by blocking chloride channels during postembryonic motoneuron development

### **1.3 References**

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