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**Impact of network activity on the axon initial segment during
development and after pathophysiological insult**

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The axon initial segment (AIS) is an axonal microdomain responsible for AP initiation and the maintenance of neuronal polarity. The master scaffolding protein of the AIS is ankyrinG (ankG), binding voltage-gated ion channels in the membrane and tethering them to the actin cytoskeleton via β IV-spectrin. Recent studies have highlighted a striking feature of the AIS, namely its ability to impact single neuron function by undergoing structural and functional plasticity in response to changes in network state. It is thought that AIS plasticity aims at constraining a single neuron's activity at a physiological optimum within a functional network and seems to be regulated in a homeostatic manner. In support of this hypothesis, several studies have demonstrated developmental AIS plasticity, an intriguing concept of neuronal remodeling during important phases of postnatal maturation termed 'critical periods'. Critical periods represent defined windows during early postnatal development when emerging neuronal circuitry can undergo significant remodeling in response to experience.

To date, these remodeling events have primarily been described for the somatodendritic domain of excitatory neurons. Strikingly, recent studies in sensory cortices (for example primary visual and somatosensory cortex) have shown that the axonal domain with the AIS undergoes similar critical period plasticity: AIS develop following a triphasic maturation pattern. They elongate until the emergence of a critical stimulus such as eye opening for the visual cortex or active whisking for the somatosensory cortex. This onset of presynaptic input leads to over-excitation of maturing cortical neurons, which compensate this increased network activity with a shortening of the AIS. Upon neuronal maturation at the end of the critical period, AIS re-elongate until they reach their physiological optimal length. Given this remarkable ability of AIS to undergo periods of structural plasticity, the aim of this thesis was to elucidate developmental and structural AIS plasticity in different model systems of altered network activity.

Project I focused on characterizing the emergence of an AIS in developing neuronal progenitor cells as a surrogate parameter for neuronal development in the piriform cortex. The neuronal progenitor cells, so called tangled cells residing in layer II of the piriform cortex, are post-mitotic and immature. Although the piriform cortex is not a neurogenic niche, tangled cells have the ability to develop into mature glutamatergic principal neurons (complex cells). These complex cells are structurally integrated into the surrounding neuronal network. However, it remained to be elucidated whether complex cells are functionally integrated into the pre-existing neuronal network. When does the AIS emerge in developing tangled cells and does the AIS undergo developmental plasticity? Immunofluorescence indicates that during tangled cell development, the AIS only emerges at the stage of complex cells and could be used as a surrogate parameter to identify them. Interestingly, the AIS of complex cells is remarkably shorter than the AIS of surrounding principal neurons in the piriform cortex. Moreover, both, complex cell AIS and the AIS of the surrounding principal neurons, elongate during aging. Electrophysiological whole-cell patch-clamp measurements in acute slices revealed that mature complex cells indeed integrate functionally into the pre-existing neuronal network. Interestingly, they encompass distinguishing physiological properties compared to principal neurons in the piriform cortex: Complex cells have different passive (e.g. membrane potential, membrane capacitance) and active (e.g. action potential, rheobase) electrophysiological properties. In particular, complex cells only receive GABAergic pre-synaptic input, whereas native principal neurons receive both, glutamatergic and GABAergic pre-synaptic input.

Project II focused on developmental and structural AIS plasticity in the primary motor cortex (M1). To date, it was not understood whether developmental AIS plasticity in non-sensory cortices differs from

the triphasic maturation pattern in sensory cortices. Interestingly, in layer 2/3 and 5 AIS in M1 show a monophasic elongation until the end of the observation period (P150). This difference could be explained with the lack of distinct critical periods for cellular plasticity in postnatal non-sensory cortices. Structural AIS plasticity has largely been demonstrated *in vitro* or in sensory cortices *in vivo*. Thus, another aim of this project was to elucidate whether the alteration of neuronal output properties influences the structural AIS plasticity. To address this question, we used an injury model where the corticospinal tract was axotomized bilaterally at cervical level 4 of the spinal cord. Axotomized neurons were visualized using the retrograde tracer fluorogold. Another critical, yet unanswered question was how cortical remodeling of the M1 network, which had been discovered based on functional imaging in human patients, might work at a single cell level. To address these issues, we used behavioral assessments, immunofluorescence, patch-clamp whole-cell recordings in acute slices, and 3D remodeling in different layers of M1. After bilateral axotomy of the corticospinal tract, lesioned animals develop a specific phenotype. Axotomized neurons neither die, nor show signs of atrophy or cell swelling. Interestingly, AIS from lesioned animals exhibit striking plasticity in layers 2/3, 5 and 6. Furthermore, only AIS in layer 5 lose a significant amount of GABAergic axo-axonic synapses. Likewise, a reduced expression of parvalbumin could be observed in layer 5, indicating significant alterations to interneuron populations as well. The observed remodeling after axotomy is accompanied by layer-specific changes in excitability in layer 5 and 6 in lesioned animals.

Taken together, this thesis provides strong evidence for different facets of developmental and structural AIS plasticity. The emergence of an AIS in developing neuronal progenitor cells is a sufficient surrogate parameter for neuronal development and network integration. Moreover, we showed that AIS in non-sensory cortices develop following a different maturation pattern compared to sensory cortices. The alteration of neuronal output properties using a spinal cord injury model provides evidence for both, structural AIS and cellular plasticity of the axotomized neurons and a remodeling of the surrounding neuronal network in a layer-specific manner. Thus, structural AIS plasticity encompasses more than maintaining neuronal excitability in the sense of homeostatic plasticity after alteration of neuronal output properties. In addition, AIS plasticity seems to play an important role for the computation of altered neuronal output properties and layer-specific cellular remodeling processes after a pathophysiological insult.