Research Overview

Upon completion of mitosis, neocortical and CA1 pyramidal neuron progenitors lose the apico-basal polarity of their neuro-epithelial ancestors and assume an un-polarized, multipolar morphology. The neurons re-establish polarity by becoming bipolar with two neurites having a leading and trailing orientation. Formation of bipolar polarity marks axon and apical dendrite specification - apical dendrite will develop from the leading process whereas the trailing process will become the axon. Studies on axon specification have dominated efforts to elucidate neuronal polarization, yielding an understanding of the underlying mechanisms. These studies have established a prevailing view that axon specification precedes and instructs neuronal polarization and is necessary for dendrite development. This notion is based primarily on findings in cultured neurons and prevailed because in these neurons, when axon development is prevented, the neurons are un-polarized and apparently have no dendrites. This notion is supported by recent findings *in vivo* where the axon is specified first from one neurite of the multipolar neuron in majority of cortical pyramidal progenitors. However, our work as well as other studies have shown that prevention of axon formation in developing pyramidal neurons does not interfere with leading process formation or the subsequent development of the apical dendrite. Furthermore, in a significant number of pyramidal progenitors, leading process formation may precede trailing process development. These findings suggest that in the presence of precisely organized extracellular cues in developing pyramidal neurons *in vivo*, localized intrinsic signaling for apical dendrite polarization might also assemble independently of axon-determining events. These mechanisms may operate whether axon formation precedes or follows polarity establishment.

We propose that distinctly higher cyclic GMP (cGMP) generated via localized assembly of a cGMP production machinery at the leading edge of developing pyramidal neurons, promotes bipolar polarity, leading process formation, and apical dendrite development. Using state of the art *lifetime decay* FLIM-FRET cGMP measurements in mouse developing pyramidal neurons in acute slice, combined with cutting edge genetic approaches, and localized manipulation of cGMP production with a genetically encoded optogenetic probe, this study is designed to determine the spatio-temporal regulation of cGMP during polarity establishment and apical dendrite development, and to identify its mechanistic basis in developing pyramidal neurons *in vivo*.

Our specific experimental aims are:

We propose that localized cGMP production at the leading process is assembled on a scaffold protein Scribble, which binds and links intrinsic cGMP synthesizing enzymes to extracellular Semaphorin3A cue. We will determine whether localized cGMP is generated across the developing pyramidal neuron during bipolar polarity and apical dendrite development using *lifetime decay FLIM-FRET cGMP measurements* (Aim #1). We will define a cGMP-synthesizing complex assembled at the leading edge and examine whether it mediates extrinsic Sema3A cue to cGMP increase. We will investigate a kinesin motor mediated localization of the complex. We will determine whether the complex regulates cGMP increase, bipolar polarity, and apical dendrite development (Aim #2). We will directly determine the role of localized rise in cGMP in early apical dendrite morphogenesis using light-induced optogenetic manipulation of cGMP in developing pyramidal neurons in acute slice (Aim #3).

Aim #1. We will examine a hypothesis that localized cGMP is generated across the developing pyramidal neuron to regulate bipolar polarity and apical dendrite development. We will directly measure cGMP in developing pyramidal neurons with genetically encoded cGMP FRET sensors expressed in embryonic cortical and CA1 pyramidal progenitors via *in utero* electroporation, using *lifetime decay FLIM-FRET cGMP measurements* in acute embryonic and early postnatal brain slice, and determine whether higher cGMP is generated at the leading process / nascent apical dendrite. We have established the slice lifetime decay FLIM-FRET imaging and provide critical preliminary findings in support of our hypothesis and its methodological feasibility.

Aim #2. We will investigate a localized cGMP-synthesis complex assembled on the scaffold protein Scribble. Our preliminary data show that Scribble associates with cGMP synthesis enzyme sGC and the Sema3A co-receptor PlexinA3. Combining FLIM-FRET imaging with cutting edge genetic manipulations to disrupt the complex, we will investigate whether the scaffold is localized to and localizes cGMP production at the leading edge of developing pyramidal neurons, and if it links extrinsic Sema3A-PlexinA3 cue to cGMP increase. We will examine whether a kinesin motor localizes the cGMP-complex to the leading process. We will determine whether the complex regulates bipolar polarity and apical dendrite development. We provide extensive preliminary data in support of this proposal.

Aim #3. We will determine whether localized light-induced cGMP increase in developing pyramidal neurons in slice using a genetically encoded optogenetic probe (BeCyclOp), can direct and manipulate apical dendrite development. We will further determine whether the critical role of the Scribble-complex in dendrite development is attributed to cGMP. These studies will determine the direct role of cGMP in early apical dendrite morphogenesis in pyramidal neurons *in vivo*.