Doctoral (Ph.D.) thesis

DEVELOPMENTAL DETERMINANTS OF SYNAPSE-SPECIFIC RETROGRADE SIGNALING IN CORTICAL NEURONAL NETWORKS

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INTRODUCTION

The two major types of neurons within the cerebral cortex were described more then hundred years ago. Inhibitory GABAergic interneurons are outnumbered by excitatory pyramidal cells by approximately 10-fold. An appropriate classification of interneuron subtypes that precisely defines, e.g., specific arrangements in their placement, cytoarchitecture, neurochemical and molecular diversity, discharge properties and connectivity are still subject of a hot and ever evolving debate. During the past decades, extensive research has been directed towards understanding organizing principles of interneuron diversity in the forebrain: we now have a refined mechanistic concept describing the birthplaces of specific interneuron subclasses, their migratory routes, and consolidates governing principles of how interneurons become molecularly and functionally diverse during embryogenesis and the early postnatal period. Interneuron diversity underpins a fundamental plasticity of neuronal networks that is a keystone of neuronal adaptation and information processing.

Retrograde signaling is the phenomenon when a bioactive messenger released from a post-synaptic neuron travels across the synaptic cleft and engages its cognate presynaptic receptors localized to the pre-synaptic terminal. Retrograde signaling is critical to maintain the plasticity of many synapses in the brain. Retrograde messengers, including endocannabinoids (eCBs), glutamate, γ-aminobutyric acid (GABA) and brain-derived neurotropic factor (BDNF), have been found to exert fundamental roles during neuronal development thus fuelling the hypothesis of a functional continuum for signaling molecules that integrates key milestones of fetal synaptogenesis and postnatal synaptic maintenance.

This thesis addresses how neuroanatomical arrangements supporting retrograde signaling coincide with the synapse-specificity of these signaling cassettes. Moreover, I describe the identification of primary neurodevelopmental functions

of endocannabinoids on cortical interneurons. Overall, this thesis demonstrates that the developmental assembly and maintenance of the enduring functional integrity of cortical neuronal networks is controlled by morphogens that act in a highly compartmentalized fashion to generate not only neuronal but also corresponding synapse diversity in the brain.

PUBLICATIONS INCLUDED IN THIS THESIS

I. Harkany T, Dobszay MB, Cayetanot F, Härtig W, Siegemund T, Aujard F and Mackie K, Redistribution of CB_1 cannabinoid receptors during evolution of cholinergic basal forebrain territories and their cortical projection areas: comparison between grey mouse lemur (*Microcebus murinus*, Primates) and rat. *Neuroscience* (2005) 135:595-609.

II. Harkany T, Härtig W, Berghuis P, Dobszay MB, Zilberter Y, Edwards RH, Mackie K, Ernfors P. Complementary distribution of type 1 cannabinoid receptors and vesicular glutamate transporter 3 in basal forebrain suggests input-specific retrograde signaling by cholinergic neurons. *Eur J Neurosci* (2003) 18:1979-1992.

III. Harkany T, Holmgren C, Härtig W, Qureshi T, Chaudhry FA, Storm-Mathisen J, Dobszay MB, Berghuis P, Schulte G, Sousa KM, Fremeau RT Jr, Edwards RH, Mackie K, Ernfors P, Zilberter Y. Endocannabinoid-independent retrograde signaling at inhibitory synapses in layer 2/3 of neocortex: involvement of vesicular glutamate transporter 3. *J Neurosci* (2004) 24:4978-4988.

IV. Berghuis P*, Dobszay MB*, Sousa KM, Schulte G, Mager PP, Härtig W, Görcs TJ, Zilberter Y, Ernfors P, Harkany T. Brain-derived neurotrophic factor controls functional differentiation and microcircuit formation of selectively isolated fast-spiking GABAergic interneurons. *Eur J Neurosci* (2004) 20:1290-1306.

V. Grumelli C, Berghuis P, Pozzi D, Caleo M, Antonucci F, Bonanno G, Carmignoto G, Dobszay MB Harkany T, Matteoli M, Verderio C, Calpain activity contributes to the control of SNAP-25 levels in neurons. *Mol Cell Neurosci* (2008) 39:314-323.

VI. Berghuis P, Dobszay MB, Wang X, Spano S, Ledda F, Sousa KM, Schulte G, Ernfors P, Mackie K, Paratcha G, Hurd YL, Harkany T. Endocannabinoids regulate interneuron migration and morphogenesis by transactivating the TrkB receptor. *Proc Natl Acad Sci USA* (2005) 102:19115-19120.

*denotes equal contribution

METHODS

Multiple immunofluorescence labeling

Neuroanatomical studies at the light microscopy level were predominantly performed by multiple immunofluorescent labelling. Image analysis was carried out by using high-resolution confocal laser scanning microscopy (*Paper I-VI*).

Whole-cell patch clamp electrophysiology

Single or dual whole cell patch-clamp techniques were utilized to study interneuron maturation and phenotypic differentiation *in vitro* (*Papers IV-VI*) and to dissect retrograde synaptic signaling at inhibitory terminals between fast-spiking (FS) GABAergic interneurons and pyramidal cells in layer 2/3 of the neocortex (*Paper III*).

In vitro studies

Experiments on cultured interneurons at high purity were undertaken to determine molecular characteristics of perisomatic basket cells. The availability of cell surface markers selectively expressed by interneuron subsets of study allowed establishing target-specific cell isolation (TSI) as a procedure of choice to select GABAergic interneurons. Briefly, TSI allows enrichment of a desired neuronal subtype and subsequent establishment of sub-population enriched cultures. We took advantage of the selective expression of the Kv channel subunit 3.1b by FS cells (*Paper IV, V*) and the early expression of CB_1Rs by cholecystokinin-containing basket cells (*Paper VI*) to dissect molecular, morphometric, and biophysical parameters of these cell types.

AIMS

The synapse specificity and correspondingly refined molecular mechanisms subserving retrograde signaling at cortical synapses prompted us to study whether a link between neurodevelopmental mechanisms of synaptogenesis – dynamic signaling between pre- and postsynaptic targets to form synapses - and the molecular substrates of retrograde signaling at mature synapses exists. We postulated that the signaling competence of some key morphogens expressed in the fetal telencephalon and released from immature dendrites to attract afferent pathways to form endures into the postnatal maturation of synapses. Particularly, we hypothesized that if the dendritic release site for these neuroactive substances is retained by postsynaptic neurons then such factors will ultimately function as retrograde messengers.

Here, I have focused on complementary studies using perisomatic basket cell subtypes given the striking differences in their molecular fingerprints and developmental programs.

In the adult telencephalon, I set out to study:

- CB_1 cannabinoid receptor (CB_1R) distribution in cholinergic basal forebrain and neocortex with particular emphasis on interspecies differences and on the recruitment of CB_1Rs to excitatory and inhibitory cortical synapses (*Paper I-II*),
- the molecular signaling network underpinning retrograde synaptic signaling between fast-spiking, CB_1R-negative interneurons and pyramidal cells in layer 2/3 of the neocortex (*Paper III*).

In the developing cerebrum, I addressed:

- whether a novel method allowing subset-specific isolation of GABAergic interneurons from embryonic cerebrum can be established to help characterizing FS interneuron development *in vitro*, and addressing BDNF requirements of FS cells during acquiring the capacity to generate high-frequency action potential trains (*Paper IV*),
- how intrinsic calpain activity contributes to the developmental regulation of synaptosomal-associated protein of 25 kDa (SNAP-25) levels in FS interneurons (*Paper V*),
- the developmental significance of CB_1R expression by immature cholecystokinin-positive cortical interneurons (*Paper VI*),
- whether eCB signaling instructs interneuron migration and morphological differentiation by interacting with developmentally-organized neurotrophin signaling cassettes (*Paper VI*).

RESULTS

Developmental assembly and maintenance of the enduring functional integrity of cortical neuronal networks is controlled by a vast array of diffusible factors. Studies involved in my thesis provide evidence that:

- organizing principles of CB_1R-containing neurons and their terminal fields within the basal forebrain are evolutionary conserved between rodents and prosminian primates (*Paper I*),
- the expansion and cytoarchitectonic differentiation of neocortical subfields in primates is associated with differential cortical patterning of CB_1R-containing subcortical and intracortical afferents (*Paper I*),
- endocannabinoid and glutamate-mediated retrograde signaling cascades contribute to the control of the plasticity of non-overlapping synapse populations in isocortical and basal forebrain territories (*Paper II*),
- a single neuron may be capable of expressing components of many retrograde signaling systems and recruit these to spatially-defined dendritic domains. However, recruitment of retrograde signaling networks is always synapse specific and is defined by a developmental interplay between pre- and post-synaptic neurons (*Paper III*),
- glutamate may act as a retrograde messenger when released in quanta from subsynaptic dendrites to modulate the efficacy of FS cell afferents (*Paper III*),
- target specific isolation is an adequate tool to enrich interneuron subtypes *in vitro* to dissect molecular, cell biology (signalling) differences amongst interneuron subclasses (*Paper IV-V*),

- endocannabinoids play unexpectedly fundamental roles during formation of the cerebral cortex (*Paper VI*).