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CaV1.3 signaling complexes and epigenetic mechanisms induced by Ca2+ channel to nucleus signaling

Aim 1: Characterizing the role of Cav1.3 membrane expression and the consequences of Cav1.3 dysregulation on dendritic stability and remodeling

Alternative splicing of Cav1.3 gives rise to a long (Cav1.3L) and two short (Cav1.342A; Cav1.343S) C-terminal variants, which differ with respect to the activation voltage range and Ca2+ conductance (Singh et al., 2008). Both long and short splice variants are expressed in substantia nigra pars compacta (SNc) and medium spiny (MSN) neurons (Olson et al., 2005) and Cav1.3 has thus been suggested to be involved in the pathophysiology of Parkinson's disease. The long C-terminal splice variant Cav1.3L has been shown to interact with erbin, densin-180, and shank, which have all been implicated in the regulation of neuronal morphology and spine integrity. Therefore we investigated targeting properties and neuronal membrane expression of Cav1.3 splice variants in cultured hippocampal neurons. Furthermore we addressed the question as to whether and how the interaction with the PDZ-domain containing proteins partners erbin, densin-180, and shank1B affect Cav1.3 membrane expression and dendritic spine stability. Using expression of extracellular epitope-tagged Cav1.3 splice variants in cultured hippocampal neurons we could demonstrate the following findings.

1. Both long as well as short Cav1.3 isoforms revealed a clustered distribution on soma, dendrites and dendritic spines. This suggests that the distal C-terminus is not responsible for the clustered surface expression of Cav1.3.

2. The short splice variants of Cav1.3 (Cav1.342A and Cav1.343S) had significantly reduced surface expression, indicating a role of the distal C-terminus in membrane trafficking or complex stability of Cav1.3.

3. Coexpression of shank and densin reduced surfaced expression level of Cav1.3L, but this effect is diminished in C-terminus splice variants lacking interaction domain. This is in contrast to Cav1.2, which showed significantly increased surface expression upon coexpression of densin.

4. Finally, preliminary experiments indicate that expression of PDZ-domain proteins densin and erbin destabilize dendritic spines. This effect is also evident upon coexpression of CaV1.3L, however, not with the other neuronal L-type channel Cav1.2.

Currently we are analyzing and quantifying the effects of densin, erbin and shank coexpression with Cav1.2 and Cav1.3 splice variants on the dendritic spine stability. Furthermore, we established electrophysiological recordings of cultured hippocampal neurons and will thus analyze the consequences of the aforementioned protein interactions/coexpressions on neuronal excitability and synaptic function. This will allow us to relate observed effects on dendritic spine stability to neuronal function and ultimately allow predictions which Cav1.3 splice variant is involved in dendritic spine integration and destabilization in GABAergic neurons in the substantia nigra in Parkinsons disease.

Aim 2: Identifying the role of Ca2+ channel □4b subunit nuclear targeting in epigenetic histone modifications (collaboration with Lusser)

Published data by us and others (Subramanyam et al., 2009; Hibino et al., 2003) indicated that the □4b subunit is targeted to the nucleus, where it might be involved in activity-induced gene regulation. A recent publication by a competing group (Tadmouri et al,. 2012) identified □4 association with a nuclear repressor complex including PP2A, B56□, HP1□ and tyrosine receptor □ that regulates histone modification and tyrosine hydroxylase gene expression in a □4-subunit dependent manner. Therefore we reoriented the focus of our Aim 2 to search for new interacting enzymes, regulatory proteins, and genes differentially expressed in a □4 isoform-specific manner. For this purpose we performed HAT, HMT, and HDAC activity assays on nuclear □4 immunoprecipitates (although so far without success) and we will analyze the precipitates with Mass-Spectroscopy. Unexpectedly we discovered a novel third splice variant of □4 (□4x) which is highly expressed in the cerebellum. To analyze the specific neuronal targeting properties and potential splice variant-specific effects on gene regulation, we established neuronal cultures from the lethargic (□4-null) mouse reconstituted

individually with each of the □4 subunit variants (□4a , □4b , □4x). Immunofluorescence localization studies indicate that unlike □4b the new □4x lacks nuclear targeting properties, but is the dominant □4 variant in the distal axon, indicative of a presynaptic function. Quantitative RT-PCR analysis indicates that mature mouse cerebellum primarily express □4a and □4x, whereas □4b is poorly expressed. Affimetrix expression profiling of RNA preparations from the differentially reconstituted cultures (□4a, □4b, □4x) is near completion. A publication on the expression and targeting of the splice variants is in preparation. The outcome of the ongoing screening activities (enzyme activity, proteomics, genomics, and ncRNA, see Aim 3) will determine whether and how this aim will be further pursued in future.

Aim 3: Exploring the involvement of ncRNAs in neuronal development, and homeostatic and Ca2+ channel-induced plasticity

Non-conding RNAs (ncRNAs) are emerging as key modulators of cellular functions by specifically enhancing or repressing gene transcription. There is increasing evidence that specific ncRNAs are also involved in neuronal functions such as dendritic spine morphogenesis, synaptic plasticity, neuronal development and disease including Alzheimer's and Parkinson's disease (Saba and Schratt, 2010). On a larger scale it is still not fully undestood which ncRNAs are expressed during neuronal development and how they are linked to neuronal activity and Cav dependent signaling events. Here we employ the neuro-ncRChip developed in P11 (Hüttenhofer) to provide novel insights into ncRNA expression. Due to the developments of the ongoing projects in our lab (see Aim 2) as well as in the Singewald and Hüttenhofer labs we will start analyzing the ncRNA profiles of hippocampal and cerebellar tissue of developing (3 week-old) and adult (3 month-old) calcium channel subunit β4 null mutant mice lethargic in comparison with littermate controls. The SFB consortium provides preliminary increasing evidence that β4 is involved nuclear functions (Aim 2) and that it is differentially expressed in paradigms for anxiety related disorders in mice (fear extinction, Singewald). Besides this, very recent and preliminary findings (Hüttenhofer) suggested the β4 subunit as a potential target of a newly identified and yet not fully characterized ncRNA.