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The role of LTCC in adult neurogenesis

Aim 1: Determine the cellular distribution of Cav1.2 and Cav1.3 within the hippocampal and subventricular neurogenic niche.

To investigate the presence and expression patterns of LTCCs within the neurogenic niches, we performed detailed immunohistological analyses of the two main brain LTCC isoforms, Cav1.2 and Cav1.3, in adult C57BL/6 mice. Using a commercially available antibody against Cav1.2, we could detect expression of this channel subtype within the main neurogenic regions, i.e. the dentate gyrus and the subventricular zone. Co-stainings with cell-type specific markers revealed Cav1.2 expression predominately in mature neurons, while neuronal progenitor cells were only rarely positive for Cav1.2. Further co-localization studies are currently running to examine Cav1.2 expression in additional cell types composing the neural stem cell niches, e.g. neural stem cells, astrocytes or microglia.

Due to the absence of reliable Cav1.3 antibodies, we made use of Cav1.3-EGFP$^{switch\ +}$ mice (provided by Bartsch via P02/Striessnig-Koschak) to detect the cellular distribution of Cav1.3 in the neurogenic niches. Co-labelling analyses of EGFP^{+} cells, which indicate Cav1.3 expression, with cell-type specific markers revealed abundant EGFP expression in neural stem cells and mature neurons, and weak staining in astrocytes. EGFP expression has not been detected in neuronal progenitor cells, which constitute the main proliferating cell type within the neurogenic niches. This expression pattern suggests that a downregulation of Cav1.3 occurs during the expansion phase of neurogenesis, followed by a subsequent re-expression during final differentiation into mature neurons. Together, these data confirmed the expression of Cav1.2 and Cav1.3 within the neurogenic niches in the adult mouse brain, supporting an involvement of LTCCs in adult neurogenesis processes. Modulation of Cav1.2 and Cav1.3 activities may thus have a crucial impact on neurogenic responses (see `aim 3´).
Besides the expression pattern in the neurogenic regions, we have analyzed LTCC expression and their functional role in TGF-beta stimulated neural progenitors ex vivo. Here, we could nicely demonstrate that TGF-beta primes proliferating neural progenitors to electrophysiological functionality, which correlated with an induced expression of LTCCs (Kraus et al., Glia, accepted for publication). Also, in a further attempt to promote neuronal differentiation of progenitors, which might involve LTCCs, we identified novel cyclic prenylflavonoids as compounds that enhance neuronal differentiation and survival (Oberbauer et al., Journal of Nutritional Biochemistry, accepted for publication).

Aim 2: Quantify the impact of Cav1.2 and Cav1.3 targeted deletion within the adult neurogenic niche on the neural stem and progenitor cells behavior.

The functional relevance of single LTCC isoforms will be evaluated by selective deletion of Cav1.2 and Cav1.3 in neural stem cells and in neuronal precursor cells. To this end, we are currently mating genetically designed mouse lines allowing the conditional deletion of Cav1.2 and Cav1.3 with Nestin-CreERT line and with a Doublecortin-CreERT line, respectively. The consequences of tamoxifen-induced conditional knockout of LTCCs in neural stem cells and

neuronal progenitors on adult neurogenesis will be analysed immunohistologically. If these knockouts affect neurogenesis, behavioural analyses will be performed in cooperation with P10/Singewald, P04/Wenning-Stefanova and P05/Humpel-Marksteiner.

The start of this aim was postponed due to difficulties in breeding and limited transgenic progeny so far. Taking into consideration the long generation time of these mouse strains, we expect to complete evaluation of the effects of conditional Cav1.3 knockout in neural stem and progenitor cells by the end of 2013. Analyses concerning the impact of Cav1.2 deletion on adult neurogenesis are intended to be completed by end of 2014.

In addition, we analyzed the role of LTCCs in neuronal network activities, in particular in the activity of intrinsically active and pacemaker neurons derived form neural stem cells (Illes et al., Stem Cell Reports, under review).

Aim 3: Investigate the impact of chronic Cav1.2 and Cav1.3 inhibition or stimulation on neurogenesis.

Chronic LTCC inhibition represents a promising therapeutic approach to ameliorate the etiopathology of neurodegenerative disorders such as PD. However, possible consequences of chronic LTCC inactivation on adult neurogenesis have not been examined so far. Thus, we analysed the effects of Cav1.3 inhibition on neurogenesis rates by using Cav1.3 deficient mice provided by P02/Striessnig-Koschak and P10/Singewald. Immunohistological analyses revealed significant alterations within the neurogenic regions of adult Cav1.3$^{-/-}$ mice compared to wildtype controls. While dentate gyrus cell proliferation rate was increased in the transgenic mice, the survival rate of newly generated cells was significantly reduced and numbers of neural stem cells detected in the dentate gyrus were lower in the knockout animals.

In addition to the effects on neurogenesis, Cav1.3$^{-/-}$ mice further exhibited an altered morphology of astrocytes. We measured that GFAP^{+} astrocytes within the hippocampus of the knockout mice offered a reduced surface area compared to GFAP^{+} cells of wildtype controls. We are currently performing cell culture experiments (proliferation and differentiation assays, cell fate analyses) to examine neural stem and progenitor cell behaviour of Cav1.3 deficient mice in vitro, and to characterise the astrocytic phenotype of these mice in more detail.

Further, we are now analysing the impact of ageing on the neurogenic action of Cav1.3 inhibition. For this, the neurogenic regions of aged (12 months old) Cav1.3 $^{-/-}$ mice will be analysed immunohistologically. In a next step, we aim to perform behavioural tests (in collaboration with P10/Singewald) to examine possible implications of the observed alterations of neurogenesis and gliogenesis on behaviour of Cav1.3$^{-/-}$ mice.

To analyse the effects of Cav1.3 specific activation, Cav1.2.DHP$^{-/-}$ mice (provided by P02/Striessnig-Koschak) have been treated with the LTCC activator BAYK8644 for 7 days by P10/Singewald. Results indicate that an over-activation of Cav1.3 leads to reduced survival of newly generated cells and to a lower number of neuronal progenitor cells within the dentate gyrus. Standardized behavioural tests performed by P02/Singewald further revealed that the observed changes in neurogenesis rates are accompanied by enhanced anxiety- and depression-related behavior.

Together, our present data suggest a critical role of LTCCs in adult neurogenic processes, and should be considered for future therapeutic administration of LTCCs.

Aim 4: Evaluate the importance of Cav1.2 and Cav1.3 in the neurogenic pathological response and reactive gliosis.

Neural stem and progenitor cell behaviour is fundamentally affected in response to CNS lesions in neurodegenerative disorders such as AD and PD. This results in alterations in proliferation, differentiation and integration of newly generated cells. In transgenic AD mouse models it has been shown that immature neuronal progenitors and reactive astrocytes appear around amyloid plaques. In this context, P05/Humpel-Marksteiner have reported upregulation of Cav1.2 in reactive astrocytes around plaques.

Within this work-package, we have demonstrated that astrocyte-like DCX positive cells (DCX: marker for immature neuronal precursors) appear around amyloid plaques in transgenic AD mouse models. Remarkably, some of these DCX positive cells co-express Cav1.2. These data suggest that Cav1.2 activity may be required for neurogenic responses during pathological states and that DCX can be used as a marker for lesion-induced CNS response. We recently reviewed the different strategies to visualize the neurogenic response in vivo and reported that transgenic models expressing the bioluminescent luciferase gene under the DCX promoter are particularly efficient to the purpose (Vande Velde et al. 2012 WIREs Nanomed Nanobiotechnol).

Additional disease models associated with CNS injury, i.e. mice treated with MTPT and 6-OHDA to induce PD, will be used to monitor the reactions of neural stem and progenitor cells and to address the involvement of LTCCs in these reactions More specifically, the presence of DCX-expressing cells as well as a possible regulation of LTCCs will be examined. Further, the effect of treatment with LTCC blockers will be analysed in these PD models, to assess the functional role of LTCCs for the response of the neurogenic niches to CNS injury.

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