P12: Birgit Liss

Functional role of L-type Ca^{2+}-channels in dopaminergic midbrain neurons in health and disease states.

Aim 1: Defining age-dependent function and expression of LTCC subtypes in SN DA neurons and their role for ageing and neurodegeneration in Parkinson's disease (PD) mouse models.

Aim 1a: For mRNA quantification of LTCC alpha subunits from juvenile (PN14) and adult (PN90) WT mice, SN DA neurons were collected via UV-LMD for RT-qPCR analysis. We detected significantly lower levels of Cav1.2 and Cav1.3 mRNA in SN DA neurons from adult compared to juvenile WT mice. In collaboration with **P02/Striessnig-Koschak**, we currently analyze cell-specific expression of three C-terminal Cav1.3 splice variants (Bock et al., 2011). Preliminary data show that all three major splice variants are expressed in juvenile SN DA neurons.

Aim 1b: We have analyzed functional properties of adult (PN 7-8 months, G4) SN DA neurons in a mouse model of accelerated ageing (telomerase RNA component, TERC KO (Begus-Nahrmann et al., 2009)) in brain slices. Our patch clamp analysis of rostral SN DA neurons from TERC KO mice, TERC +/- mice and WT C57BL/6 mice indicate that SN DA neurons form TERC KO mice display changes in calcium handling (potentiated depolarization induced calcium waves, significantly smaller after-hyperpolarization, AHP) as well as altered K-ATP channel activity (hyperpolarization and larger basal currents, faster washout rate and stronger hyperpolarization after washout). We aim to identify the source of changes in calcium handling and to investigate the influence of the PARK7-mutation (DJ-1 KO), already known to induce increased LTCC-generated oxidative stress levels (Guzman et al., 2010), when crossed to TERC KO mice.

As proposed, we have set-up a multi-electrode array (MEA) device for recording of extracellular activity from neurons in mouse midbrain slices, in order to allow rapid screening for the effect of novel LTCC-inhibitors on SN DA pacemaker activity, without perturbation of intracellular signaling. Multi-unit signals were split into single-unit clusters and separated from noise and background activity by a spike sorting algorithm in Matlab. Due to a low signal-to-noise ratio, several experimental improvements for all steps from slice preparation to the slice-electrode-interface were necessary. In addition, we designed a custom MEA-Biochip with a better coverage of the SNc area. SN DA-like activity was pharmacologically identified by its inhibitory response to dopamine (D2-autoreceptor, D2-AR response). Our current approach results in an average yield of 12 clusters with SN DA-like activity per slice. We are currently addressing methodological drawbacks of this system, before we can proceed to reliable pharmacological screening experiments. We also aim to implement a model-based spike sorting algorithm, which considers additional activity-properties of SN DA neurons, in addition to waveform-based features.

Aim 2: Dissecting the role of LTCC function for the plasticity of D2-autoreceptor responses and Ca^{2+}-dependent HDAC translocation.

Aim 2a: In collaboration with **P02/Striessnig-Koschak,** we defined a novel function of LTCCs in SN DA neurons for controlling inhibitory dopamine D2-autoreceptor (D2-AR) responses: We show that D2-AR responses of SN DA neurons (in *in vitro* mouse brain slices), vary in strength during postnatal maturation, as well as in response to a transient high-dopamine states *(in vivo* cocaine injection, 15mg/kg i.p.). Rapidly desensitizing D2-AR responses of juvenile SN DA neurons could be converted to adult-like non-desensitizing responses by one dose of cocaine *in vivo*. By using pharmacological (300nM isradipine) and genetic (Cav1.3 KO, Cav1.2 $DHP^{-/-}$ tg mice) tools, we identified an underlying signaling pathway, dependent on the function of Cav1.3 LTCCs, and the downstream calcium-dependent interaction of D2-ARs with the neuronal calcium sensor 1 (NCS-1) (Dragicevic et al., submitted). While Cav1.2 $DHP^{-/-}$ mice responded like WT, the constitutive absence of Cav1.3 activity throughout development in Cav1.3 KO mice seems to prevented the postnatal juvenile WT SN DA D2-AR phenotypes, as the non-desensitizing "adult" D2-AR responses were present in SN DA neurons from both, juvenile as well as adult Cav1.3 KO mice.

Our full functional brain-slice patch clamp characterization of SN DA neurons of the Cav1.3 KO mice suggests a compensatory calcium phenotype in juvenile Cav1.3 KO SN DA neurons: The AHP of juvenile SN DA neurons from Cav1.3 KO mice was significantly larger compared to WT, while in adult mice results were opposite. The AHP in SN DA neurons depends in particular on an interplay of SK-channels, internal calcium and T-Type Ca^{2+} channels. The altered, non-desensitizing SN DA D2-AR

response of juvenile Cav1.3 KO mice could be turned into WT like, rapid-desensitizing D2-AR with a peptide (DNIP) that blocks the Ca^{2+} dependent interaction of NCS-1 with D2-ARs (Saab et al., 2009). Preliminary experiments indicate that the T-type channel blocker 941 (Tringham et al., 2012) has a similar effect as DNIP, hinting to a compensatory increased T-Type activity in juvenile SN DA neurons from Cav1.3 KO mice. We are currently performing a quantitative analysis to test if numbers of calbindin (CBd28k) positive SN DA neurons are altered in Cav1.3 KO. We will next address, if L-Dopa induces a similar Cav1.3 dependent D2-AR response as cocaine to WT SN DA neurons.

Aim 2b, D2-autoreceptor signaling itself has been shown to control HDAC-mediated gene repression (Liu et al., 2005). We have however not yet addressed HDAC expression (aim 2b).

Aim 3: Defining age-dependent LTCC subunit and HDAC expression in human SN DA neurons from post mortem brains and its potential cell-specific dysregualtion in PD.

Human *post mortem* midbrain tissue was provided from the German BrainBank (PD: n=13, 79±1 years; aged controls: n = 20, 72±1 years; younger controls, n = 7, 39±3 years). Human post-mortem brain samples are often not ideally matched with respect to age and to RNA quality (as in our case). Therefore, we have developed a mixed-effects model that allows us to distinguish between RNA-quality-, age- and disease-related effects on detected mRNA-levels of distinct samples (Schlaudraff et al, in review). While mRNA levels of Cav1.2 or Cav1.3 were not altered, we detected significantly elevated mRNA levels for NCS-1, D2-AR and GIRK2 (as well as for calbindin, CB-d28k) in human SN DA neurons from PD brains compared to age-mached controls. This strongly implies that the novel Cav1.3 function in SN DA neurons in NCS-1 mediated D2-AR control of homeostatic fine tuning of SN DA activity that we identified in mice (aim 2), might also be present in the diseased human brain.

Papers submitted:

- *Dragicevic E, Poetschke C, Duda J, Schlaudraff F, Lammel S, Schiemann J, Fauler M, Hetzel A, Watanabe M, Lujan R, Malenka RC, Striessnig J, Liss B. Cav1.3 channels control D2-autoreceptor responses via NCS-1 in Substantia Nigra dopamine neurons (in review).*
- *Schlaudraff F, Grundemann J, Fauler M, Dragicevic E, Hardy J, Liss B. Orchestrated increase of dopamine and PARK mRNAs but not miR-133b in dopamine neurons in Parkinson's disease (in review).*

Current SFB collaborations:

- *Striessnig-Koschak (P02)*

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- *Bock G, Gebhart M, Scharinger A, Jangsangthong W, Busquet P, Poggiani C, Sartori S, Mangoni ME, Sinnegger-Brauns MJ, Herzig S, Striessnig J, Koschak A (2011) Functional properties of a newly identified C-terminal splice variant of Cav1.3 L-type Ca2+ channels. J Biol Chem 286:42736-42748.*
- *Guzman JN, Sanchez-Padilla J, Wokosin D, Kondapalli J, Ilijic E, Schumacker PT, Surmeier DJ (2010) Oxidant stress evoked by pacemaking in dopaminergic neurons is attenuated by DJ-1. Nature 468:696-700.*
- *Liu JC, Baker RE, Chow W, Sun CK, Elsholtz HP (2005) Epigenetic mechanisms in the dopamine D2 receptor-dependent inhibition of the prolactin gene. Mol Endocrinol 19:1904-1917.*
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- *Tringham E, Powell KL, Cain SM, Kuplast K, Mezeyova J, Weerapura M, Eduljee C, Jiang X, Smith P, Morrison JL, Jones NC, Braine E, Rind G, Fee-Maki M, Parker D, Pajouhesh H, Parmar M, O'Brien TJ, Snutch TP (2012) T-type calcium channel blockers that attenuate thalamic burst firing and suppress absence seizures. Sci Transl Med 4:121ra119.*