Klawonn-Group: Exploring Circuits and Mechanisms of Affective State and Parkinson's Disease

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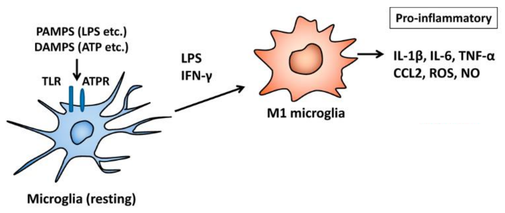
INTRODUCTION

How does the immune-system influence our mood? Why are people with inflammatory conditions or neurodegenerative diseases much more prone to develop depression? These are some of the questions we are trying to answer in the CAN-group, in the hope to uncover better treatments and tools for early diagnosis of affective disorders and neurodegenerative disease. The Circuits of Affective Neuroscience group was born December 2020 - Our research evolves around deciphering the neural circuits and immune-to-brain signaling mechanisms involved in regulating affective state during disease. Below are examples of two research lines in the group.

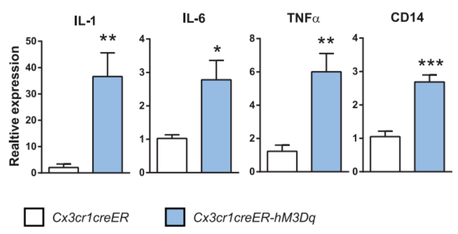
AIM
TO INVESTIGATE THE ROLE OF IMMUNE-TO-BRAIN SIGNALLING AND CIRCUIT DYNAMICS IN AFFECTIVE AND INFLAMMATORY DISEASES

Microglia – from Depression to Parkinson's Disease

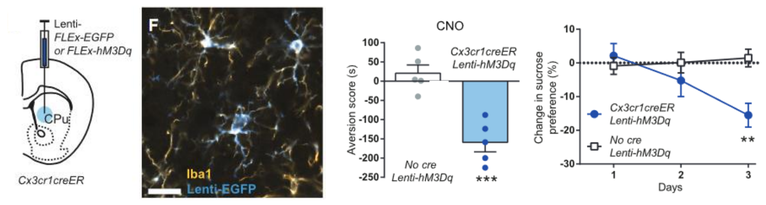


Klawonn et al. 2021, Immunity

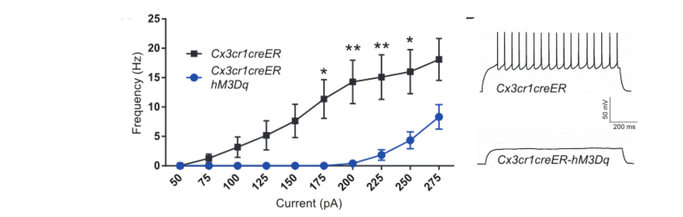
Microglia are the innate immune cells of the brain. They constitute approximately 10% of the total CNS cell population and can exist in a resting state versus an active pro-inflammatory state (M1). To explore microglia activation in affective state, we introduced a Gq-GPCR Designer_Receptors_Exclusively_Activated_by_Designer_Drugs (DREADDs) in microglia using a Lenti-viral vector in transgenic cx3cr1CreERT2 mice.



Activating Microglia using a Gq-DREADDs explicitly activated by the artificial agonist clozapine-N-oxid (CNO) leads to a pro-inflammatory signaling profile with elevated expression of cytokines, Interleukin-1, -6 and TNF-alpha, and microglia activation marker CD14 in the dorsal striatum.

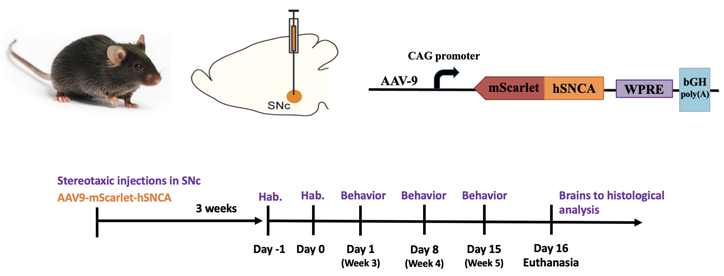


Activating microglia in the dorsal striatum of mice causes both aversion and anhedonia, as seen in conditioned place preference and sucrose preference paradigms when comparing cx3cr1CreERT2 mice with a fluorescent protein versus mice with the DREADDs construct receiving i.p. CNO (2 mg/kg).



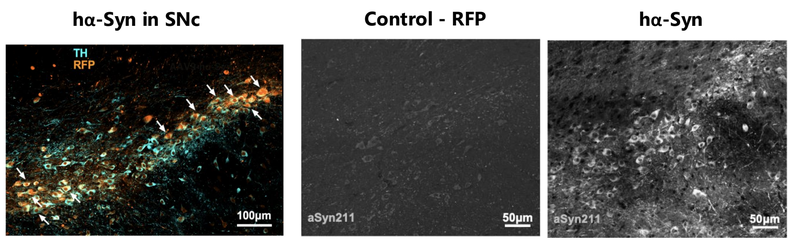
Microglia activity directly influences medium spiny neuron (MSN) excitability in the striatum. Whole cell patch clamped D1 and D2 receptor MSNs decreased their excitability upon increased current injections after microglia activation, compared to controls without DREADDs-construct.

Overexpression of human α-Synuclein PD-model



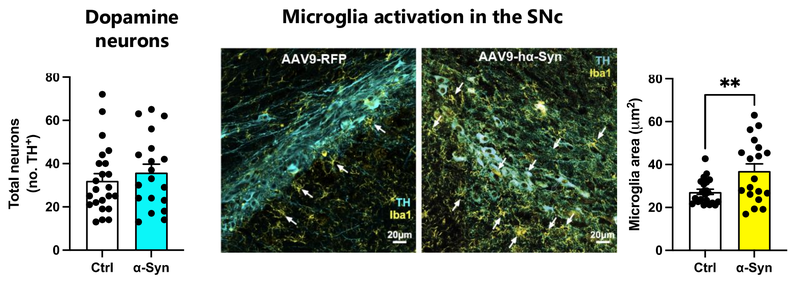
To introduce α-Syn pathology, an Adeno-Associated Virus (AAV) carrying the human α-Synuclein (hα-Syn) gene and a red fluorescent reporter protein (RFP) was injected into SNc of male mice, 8-12 weeks old.

Overexpression of hα-Syn in dopamine neurons in the SNc



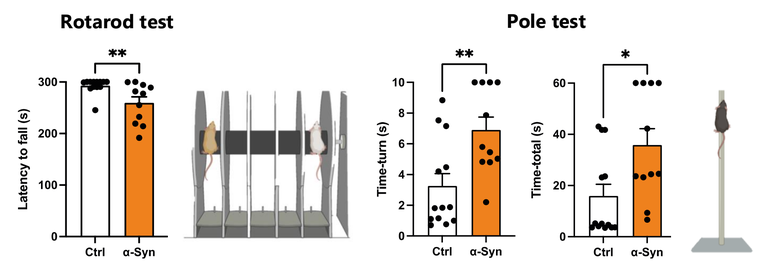
Confocal images (20x) show RFP expression (orange) in SNc dopamine neurons (cyan) following AAV9-hα-Syn-RFP transduction. There is an increase in fluorescence intensity from αSyn211-positive cells in the SNc of a mouse injected with AAV9-CAG-hα-Syn-RFP compared to one injected with AAV9-CAG-RFP.

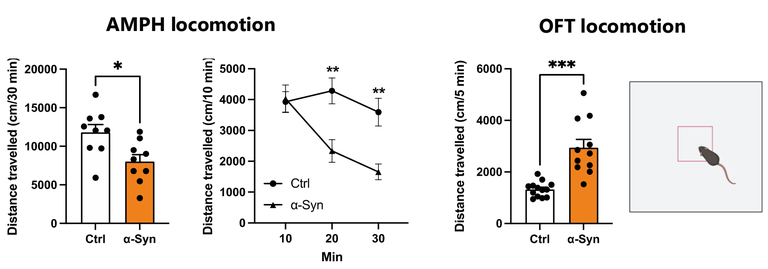
Neuroinflammation in the early Parkinson's Disease model



No differences in the number of dopamine neurons within the SNc were observed 5 weeks post-viral injection. Microglial accumulation (Iba1-positive) and increased microglial soma size were observed in the SNc of mice expressing hα-Syn compared to controls.

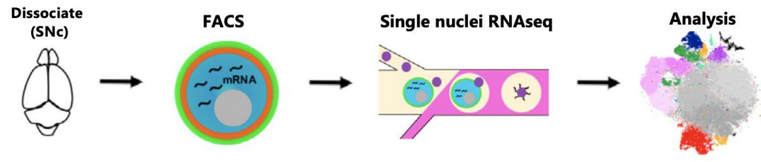
Overexpression of hα-Syn in SNc leads to motor dysfunction





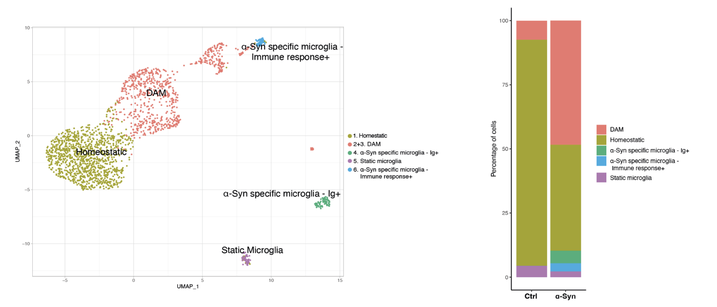
Overexpression of hα-Syn in the SNc led to impaired motor skills on the rotarod and pole tests, and reduced amphetamine (AMPH)-induced locomotion in mice. However, in the open field test (OFT), these mice showed increased activity.

Single Nuclei RNA sequencing



Single Nuclei RNA sequencing (snRNAseq) workflow to explore microglia expression profiling in our hα-Syn overexpression mouse model.

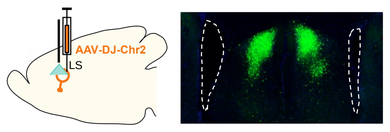
Microglia subpopulations in hα-Syn mice



Microglial nuclei with six identified microglia clusters and the relative abundance of each cluster in the midbrain in response to AAV9-RFP or AAV9-hα-Syn-RFP injections.

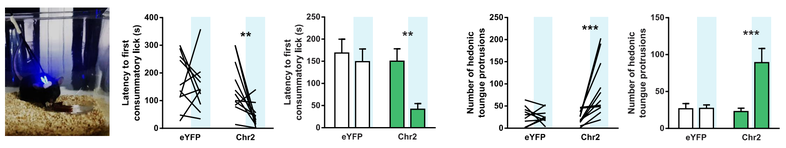
The Lateral Septum – A key regulator of mood?

In the early 50's, deep brain stimulation studies of the Lateral Septum (LS) in humans indicated it as a potential site of pleasure and positive affect. This circuitry has since then remained unexplored. In the present study, we are deciphering the role of lateral septum circuits in regulation of mood, using optogenetics and DREADDs.

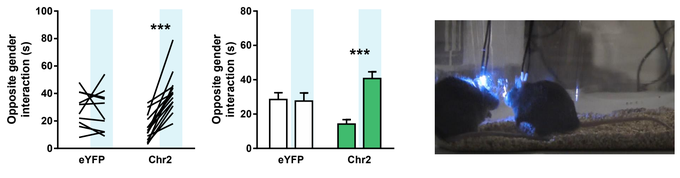


WT mice AAV-DJ-hSYN-Chr2 in LS bilateral stimulation 473 nm 20 Hz, ~10 mW

Orofacial reactivity assay – hedonic responding

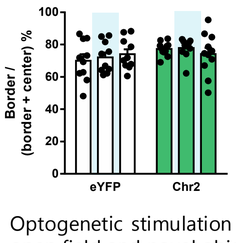


Opposite gender social interaction

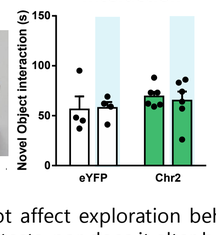


Optogenetic stimulation of LS using AAV-DJ-Chr2-eYFP in WT mice causes significantly increased hedonic responding to Nutella and opposite gender interaction in comparison to control animals (AAV-DJ-eYFP).

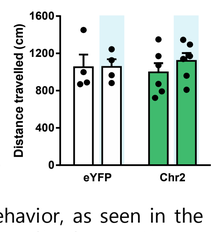
Open field test



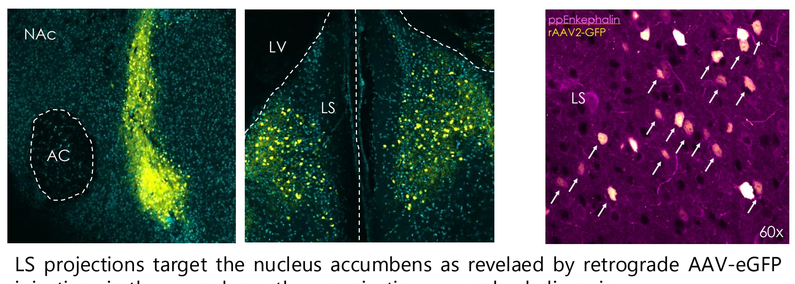
Novel object interaction



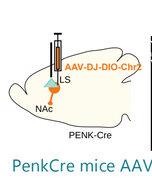
Locomotion

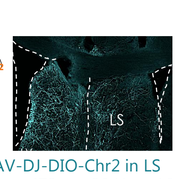


Optogenetic stimulation of LS does not affect exploration behavior, as seen in the open field and novel object interaction tests, nor does it alter baseline locomotion.



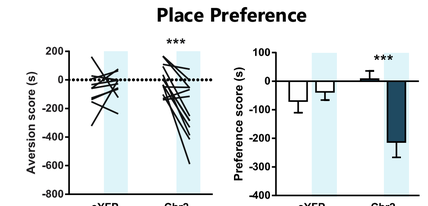
LS projections target the nucleus accumbens as revelaed by retrograde AAV-eGFP injections in the accumbens, these projections are enkephalinergic.





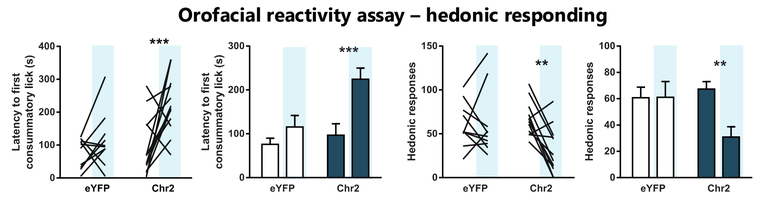
PenkCre mice AAV-DJ-DIO-Chr2 in LS bilateral stimulation 473 nm 20 Hz, ~10 mW

Place Preference

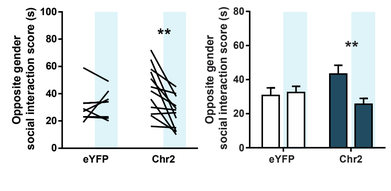


Using a Cre-dependent Chr2 strategy (AAV-DJ-DIO-Chr2-eYFP in PenkCre mice) to stimulate LS enkephalinergic neurons (or projections to the NAc) causes significant place aversion in mice.

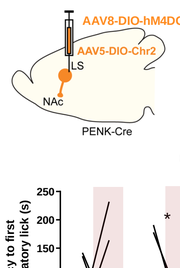
Orofacial reactivity assay – hedonic responding



Opposite gender social interaction

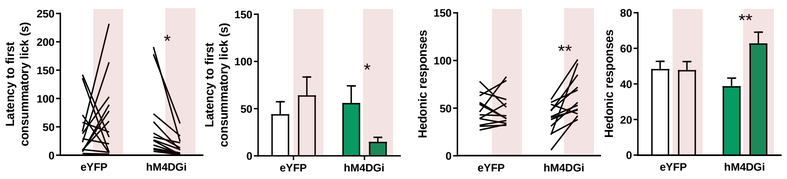


Optogenetic stimulation of LS enkephalinergic neurons causes significant decreases in hedonic responding to Nutella and reduced positive social interaction in mice, without changing novel object exploration, locomotion or anxiety levels (data not shown)



PenkCre mice AAV8-DIO-hM4DGi in LS 400 nL per site
4 mg/kg CNO or Saline i.p. 20 min prior to test

Orofacial reactivity assay – hedonic responding



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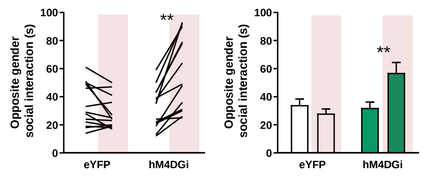

Inhibition of LS enkephalinergic neurons using the inhibitory DREADD led to a positive affective phenotype characterized by increased hedonic responding and social interaction, without influencing control-behaviors (data not shown).

IN CONCLUSION…

- **STRIATAL MICROGLIAL ACTIVATION CAUSES DEPRESSIVE BEHAVIOR IN MICE**
- **SNc MICROGLIA ACTIVATION IMPAIRS MOTORCOORDINATION**
- **LS ENKEPHALINERGIC NEURONS ARE KEY REGULATORS OF AFFECTIVE STATE**