NENS Exchange Grant – Final report

Hendrik Heiser

Home programme: MSc/PhD/MD-PhD Program and International Max Planck Research School Neurosciences, Göttingen, Germany

Host: EDNE Doctoral Program in Neuroscience, Lausanne, Switzerland

Period of stay: 17.09.2018 – 16.03.2019

"Functional connectivity analysis of the ventromedial thalamus upon remote fear memory extinction"

Aim:

The aim of my project was to clarify how the connectivity of the ventromedial thalamic nuclei contributes to its influence on remote contextual fear memory (CFM) extinction, the process underlying exposure therapy, which is the most popular treatment for anxiety and fear disorders such as posttraumatic stress disorder (PTSD). I combined retrograde viral projection labelling with activity-dependent cFos imaging during a remote CFM paradigm to examine activation of distinct efferent populations. Additionally, local chemogenetic inhibition of specific projections allowed me to gain further knowledge about the functionality of these projection neurons.

Overview:

Traumatic events can lead to the formation of some of the most enduring forms of memory that risk degenerating into a pathological state known as post-traumatic stress disorder (PTSD). The repeated exposure to reminders of the traumatic event in a safe environment can lead to the attenuation of the fearful component of trauma-related memories. This memory extinction process forms the basis of the most successful treatments for PTSD. While recall of recent memories is dependent on the hippocampus, remote memory is thought to be stored in a distributed cortical network. However, the underlying functional network of remote fear memory extinction is largely unknown. Recently, Silva et al. (2018) found that the nuclei of the ventromedial thalamus (VMT), a central hub in the remote memory network, mediates remote contextual fear memory (CFM) attenuation. Specifically, its connectivity with the medial prefrontal cortex (mPFC), hippocampal area CA1 and the basolateral amygdala (BLA) is hypothesized to be critical for remote memory extinction.

My results suggest that VMT-BLA projections are active during remote recall and extinction (Fig. 1). More importantly, mPFC-VMT projectors show increased activity only during remote extinction (Fig. 2). Furthermore, preliminary experiments suggest an impairment of remote extinction after chemogenetic inhibition of these projectors (Fig. 3). Interestingly, direct mPFC-BLA projections were not more active during the remote CFM task (Fig. 4).

Based on these findings I propose an indirect axis of mPFC-VMT-BLA to be important for remote CFM extinction rather than the direct mPFC-BLA pathway mediating recent CFM processing. This would corroborate the theory that recent and remote memories are processed differently in the brain and increases the importance on conducting research on remote memory to examine pathological fear disorders like PTSD.

Methodology and experience:

I had a scientifically productive and enriching experience in the Gräff Lab. I learned for the first time to semi-independently plan, execute, document and analyse a larger project myself. This gave me experience in evaluating the time, effort and resources necessary to conduct experiments and projects, a skill that will undoubtedly be useful in my future scientific career. Additionally, I was able to learn how to conduct long-term behavioral experiments. The paradigm for remote memory extinction developed in the Gräff lab is a powerful tool to examine long-term memory processes and can be applied in my home laboratory to extend our research on memory to remote memories. I also absolved the FELASA animal handling course and learned to perform stereotaxic surgeries on mice, a necessary skill for most neuroscientific projects.

In conclusion, my project in the Gräff lab was a very successful experience. I was able to learn several crucial skills for neuroscientists, from which I and my host laboratory will surely benefit in the future. I sincerely thank FENS and NENS for supporting me during this stay and making this unique experience possible. Thanks to the NENS Exchange grant I was able to conduct my master thesis project at an external laboratory rather than at my home university. As my home university did not support me for an external project, NENS made it possible to financially support myself during this time abroad. Conducting the project at such a renowned institute such as EPFL greatly improved not only the quality and scientific relevance of my project, but also my own cultural, social and scientific experience. I could appreciate the value of academic exchange for scientific and technical expertise, as well as expanding my academic network and cultural horizon.

The lab of Johannes Gräff with me during a hike in the Swiss Alps on top of the mountain Säntis.

Figures:

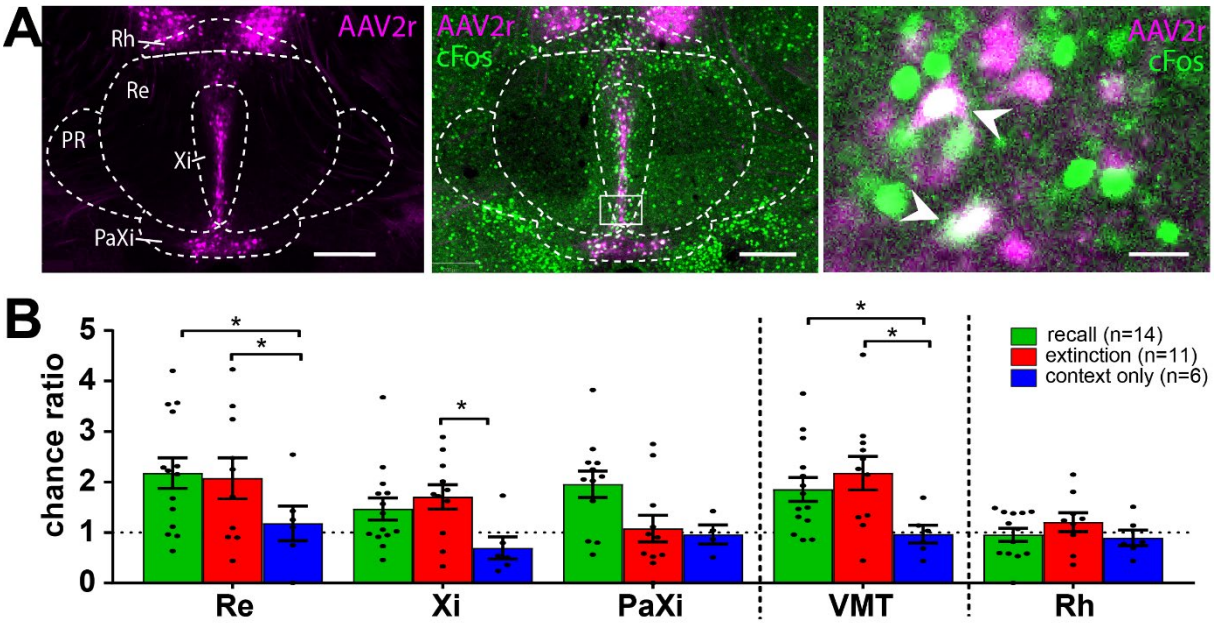

Figure 1: cFos analysis of BLA efferents in the ventromedial thalamus. (A) Retrogradely marked cells in the ventromedial thalamus (VMT) and subregions after AAV2r-injection into the BLA (left, scale=250 µm), cFos IHC staining (middle, scale=250 µm) and corresponding high-magnification zoom (right, scale=25 µm). **(B)** cFos activity levels of BLA efferents in the VMT and subregions (rhomboid (Rh) and perireuniens (PR) nuclei are not considered part of the VMT). Mice in different groups are sacrificed after initial remote recall of the memory (recall, green), after 4 days of re-exposure (extinction, red), and after the extinction paradigm without receiving foot shocks during conditioning (context only, blue). BLA efferents in the VMT show increased activity during recall and extinction (two-way ANOVA ($F(2,132)=7.96$, $P=0.0005$), Holm-Sidak post hoc).

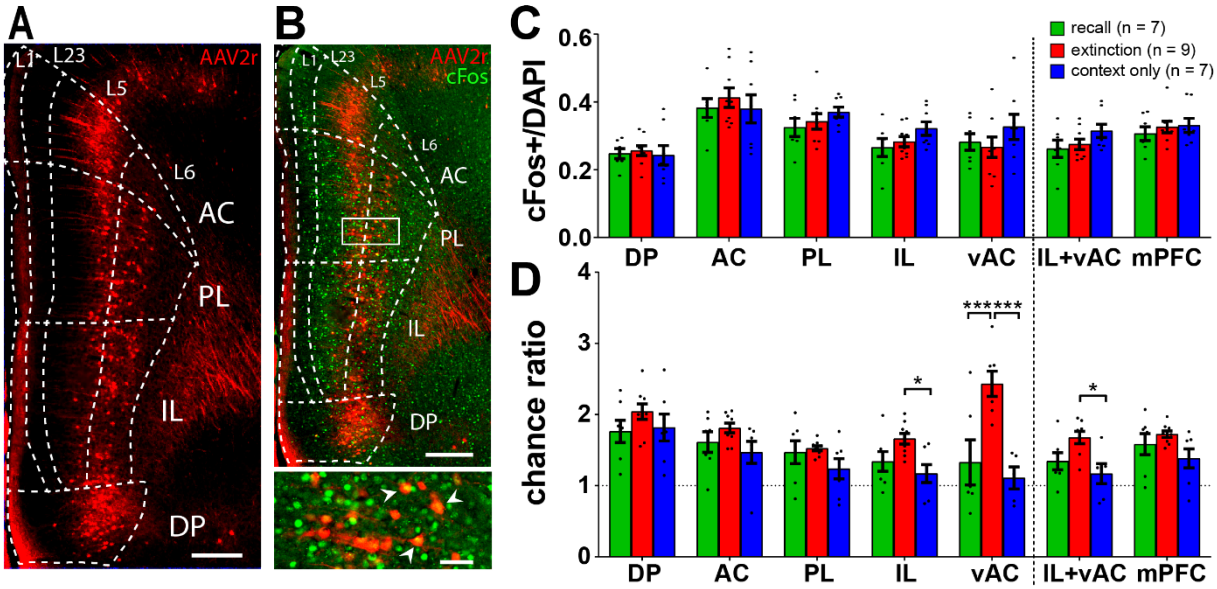

Figure 2: cFos analysis of VMT efferents in the medial prefrontal cortex. (A) Retrogradely marked cells in the medial prefrontal cortex (mPFC) and subregions after AAV2r-injection into the VMT (scale=250 µm). **(B)** cFos IHC staining (top, scale=250 µm) and high-magnification zoom of double-stained cells (bottom, scale=50 µm). **(C)** cFos expression levels in the mPFC and subregions, normalized by DAPI. No differences between behavioral groups were detected (two-way ANOVA (F(2, 147)=2.674, P=0.07). **(D)** Projection-specific cFos expression in the mPFC and subregions. VMT efferents in the infralimbic (IL) and ventral anterior cingulate (vAC) cortices show increased activity during extinction, but not recall (Two-way ANOVA (F(2,133)=26.28, P<0.0001), Tukey post hoc).

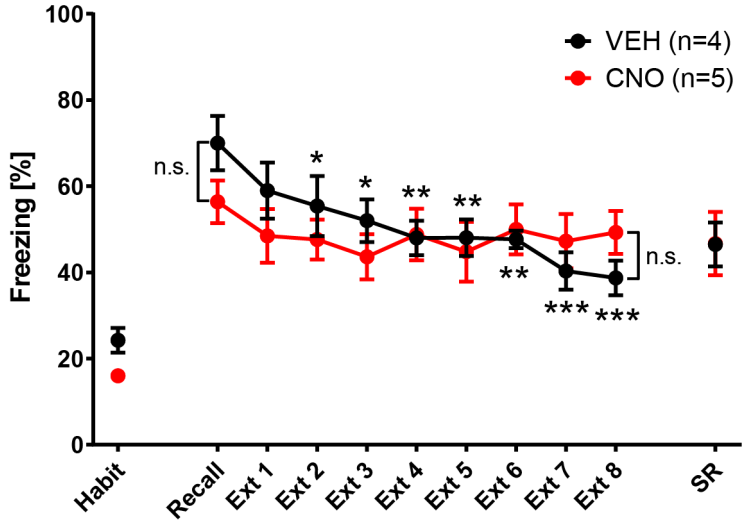

Figure 3: Chemogenetic inhibition of mPFC-VMT projectors. The inhibitory DREADD hM4Di was locally injected into the mPFC. During behavioral tests, mice received either local saline (VEH, control) or CNO (inhibition) injections into the VMT to selectively inhibit mPFC afferents. Mice in the control group showed decreasing freezing levels over time, with intact fear extinction learning. However, CNO-injected mice did not show decreasing freezing levels, thus had impaired fear extinction.

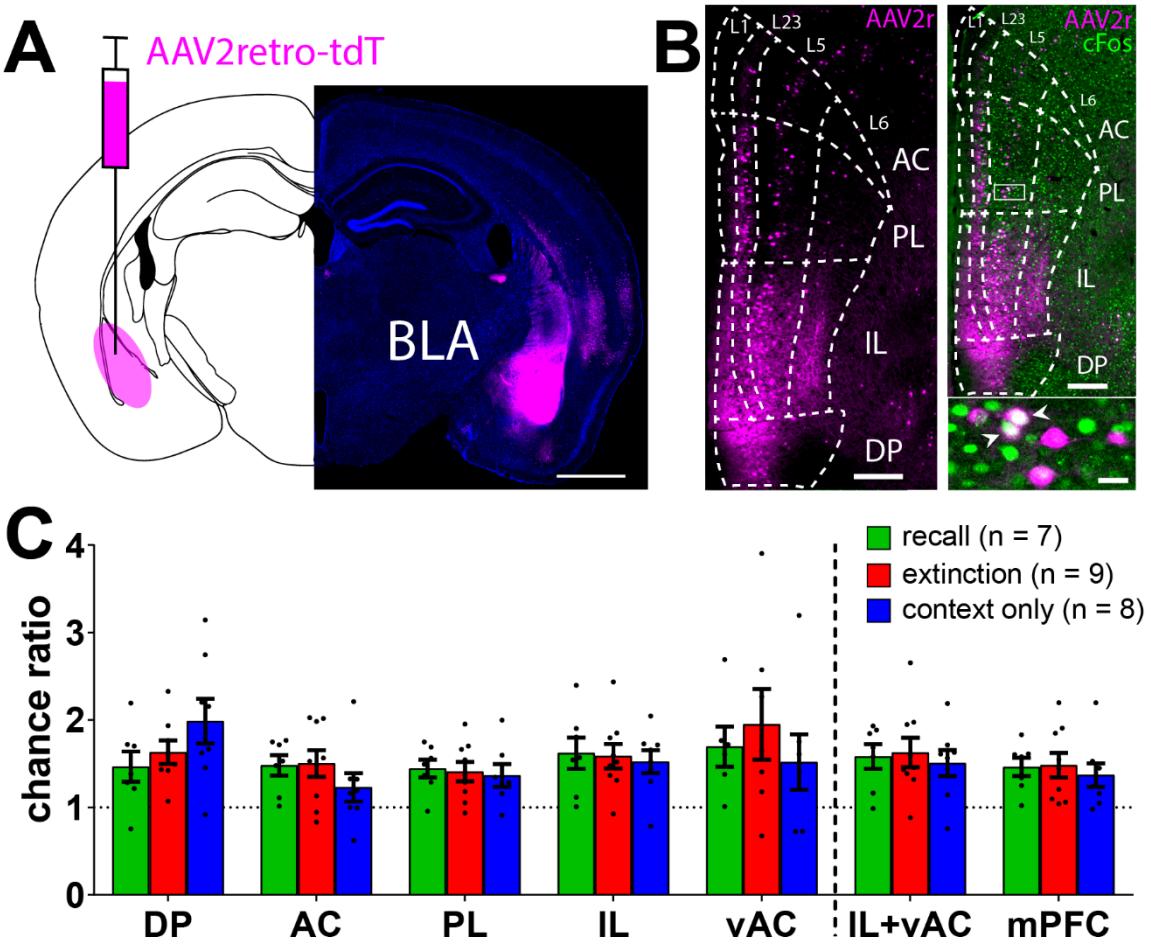

Figure 4: cFos analysis of VMT efferents in the medial prefrontal cortex. (A) Schematic representation of bilateral AAV2r injections into the BLA (scale=1mm). **(B)** Retrogradely marked cells in the mPFC and subregions after AAV2r-injection into the BLA (left, scale=250 µm), cFos IHC staining (top right, scale=250 µm) and corresponding high-magnification zoom of double-labelled cells (bottom right, scale=25 µm). **(C)** cFos expression of BLA afferents in the mPFC and subregions. No difference between behavioral groups was detected (two-way ANOVA ($F(2,141)=0.5681$, $P=0.5679$)).