NENS Exchange Grant Report

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Host Lab: Laboratory of Emotions Neurobiology (Knapska Lab), BRAINCITY, Nencki Institute of Experimental Biology PAS

Host Lab Supervisor: Prof. Ewelina Knapska

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Olfaction is one of the most evolutionary preserved senses which allows an organism to detect chemical signals in the external environment. Olfactory perception is mainly modulated by bottom-up inputs arriving in the olfactory bulb, however, top-down inputs also shape olfactory perception on a basis of prediction, expectation and memory of previous experiences (Freeman and Schneider, 1982; Kay and Laurent, 1999; Doucette and Restrepo, 2008; Mandairon and Linster, 2009; Moreno et al., 2009). One important brain region mediating strong top-down modulation on the system is the hippocampus (HPC) with direct excitatory inputs to the olfactory system (Martin et al., 2007; Aqrabawi et al., 2016). HPC has been proven to have a leading role in mediating episodic memory by many lesion and recording studies in human and non-human animals (Tulving and Markowitsch, 1998; Eichenbaum, 2017), while specifically place cells in HPC are first in line for mediating the spatial context of an event (Moser et al., 2008). With regards to HPC innervation of the olfactory system, labeling studies have demonstrated that the anterior olfactory nucleus (AON) is a principal recipient of HPC innervation (Swanson and Cowan, 1977; de Olmos et al., 1978; Haberly and Price, 1978; Van Groen and Wyss, 1990; Cenquizca and Swanson, 2007). AON is a ring-like cortical region that is divided into four regions: pars medialis (mAON), pars dorsalis (dAON), pars lateralis (lAON), and pars ventralis (vAON) (Brunjes et al., 2005).

Recently, it has been shown that HPC inputs to AON modify perception of olfactory cues and odor-guided behaviors (Aqrabawi et al., 2016), but also that HPC inputs to AON are crucial for context-driven recollection of odors and exhibit an anatomical, but also functional specificity (Aqrabawi et al., 2018). Of particular interest for our research aim are dorsal HPC to lAON unidirectional projections, since these are known to mediate association of odors with space. Additionally, optogenetic inhibition of this pathway during memory retrieval, strongly diminishes the ability of mice to recall odors with regards to their spatial distribution in a spatiotemporal episodic memory test (Aqrabawi et al., 2018). In this project we are interested in elucidating the circuit underlying exploitation of the social information about location of distant food transmitted during social interaction with a recently fed partner, a behavioral protocol developed in the laboratory of Prof. Knapska. Having already shown in the lab that HPC is activated in the recipient naïve mouse, involving remapping of place cells, we aim to decipher specifically how optogenetic manipulation of the projections from dorsal HPC to lAON can affect the utilization of socially transferred information about distant food location.

During my three-month training stay in the laboratory of Prof. Knapska in Warsaw, Poland I had the opportunity to get familiar with a great array of state-of-the-art techniques and tools used in neuroscience that are set-up and used in the Knapska Lab. Particularly, in order to carry out the proposed project, I was trained in 1. how to construct thread-attached optic fiber cannulas used for optogenetic experiments, 2. perform stereotaxic surgeries in mouse brain for the delivery of viral vectors carrying opsins in the HPC, 3. lower optic fiber cannulas in lAON and mount them with dental cement (to be used on freely-moving animals), and 4. carry out the behavioral protocol that has been established in the Knapska Lab, exploring social transfer of information about distant food and conduct optogenetics in freely-moving animals during the test.

The first step for optogenetic experiments is to assemble optic fiber cannulas for the transmission of laser light to the regions of interest. With that being said, I was trained in how to properly strip and cut optic fibers for cannulas preparation, glue them in ferrules, polish one end (through which the laser enters) and

cleavage the other end (from where the laser exits) using a diamond blade. Next, we glued them in threads used for screwing optic fibers for laser transmission. Furthermore, I was taught how to evaluate the reliability of the polishing procedure using a fiber microscope and verify proper cleavage to eliminate laser dispersion.

To begin with, all experiments were conducted in adult male C57Bl/6J animals. We first identified the stereotaxic coordinates of lAON in mouse brain by infusing Evans Blue dye in the region of interest. For the implantation of optic fiber cannulas, we modified these coordinates so that the cleaved end was placed right above lAON and hence the transmitted laser fully encompassed the region of lAON. As a first experiment, we wanted to decipher whether optogenetic activation of the halorhodopsin-carrying virus affected locomotion of mice. For this purpose, we injected bilaterally the viral construct rAAV5/CamKIIa-eNpHR3.0-eYFP (Virus Vector Core) in dorsal HPC, but also implanted self-made optic fiber cannulas unilaterally in lAON (Fig.1 A1). After allowing at least 2 weeks for viral expression, we measured the distance travelled and the average velocity of a mouse during periods without (Fig. 1 B2 and B3) or with laser transmission (Fig. 1 C2 and C3) and activation of the opsin. We used a yellow laser (wavelength = 589nm) for optogenetic activation. As shown in Figure 1, including representative tracking periods (Fig. 1 B1 and C1), equal levels of velocity and distance travelled were observed. Next, brains were fixed with 4% paraformaldehyde solution and imaged using a fluorescent microscope (Nikon Eclipse Ni-U microscope) to validate viral expression in HPC and the existence of projection in lAON (Fig. 1 A2 and A3). In Fig. 1 A1 we can see a diagram depicting our surgical procedures.

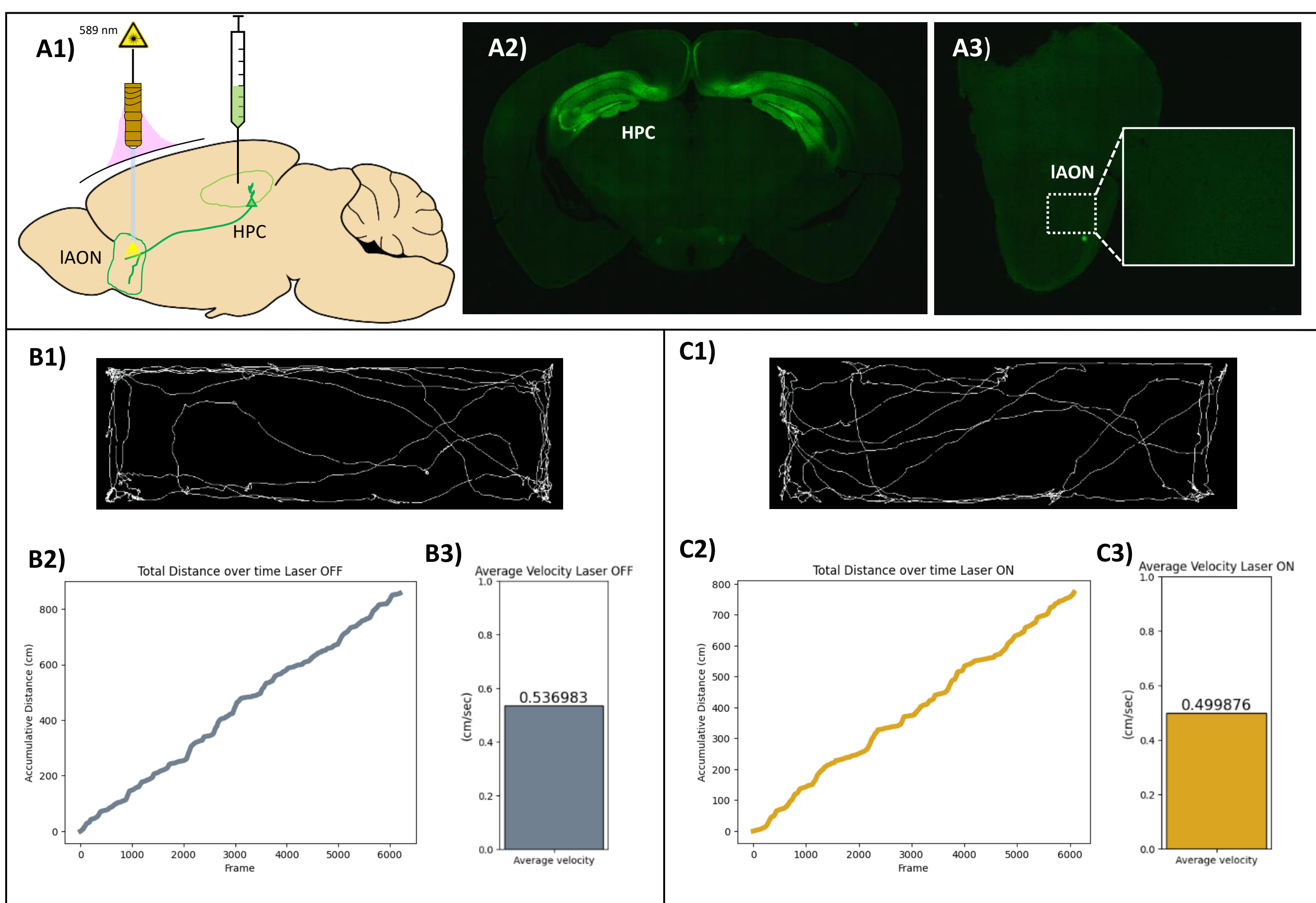

Figure 1. A1) Representative workflow of rAAV5/CamKIIa-eNpHR3.0-eYFP injection in HPC and optic fiber cannula implantation in lAON. Expression of the virus in **A2)** HPC and **A3)** lAON. **B1)** Representative tracking of the animal for 2 minutes in an open field apparatus without laser activation, and **B2)** total distance travelled (cm), **B3)** average velocity (cm/sec). **C1)** Representative tracking of the animal for 2 minutes in an open field apparatus with laser activation, and **C2)** total distance travelled (cm), **B3)** average velocity (cm/sec).

After confirming that viral injections, optic fibre cannulas implantation and photoinhibition did not alter mouse locomotion and the viral construct was expressed in regions of interest, we proceeded with viral injections in HPC and optic fibre cannulas implantation in more mice to decipher how optogenetic inhibition of HPC to lAON projections affects utilization of socially transferred information about distant food location. In a nutshell, this place preference test is conducted in a three-chamber apparatus (Fig. 2 A1). The two side chambers have distinct visual and odorant cues. Pairs of animals are composed of one animal that is the donor of information about food localization and does not receive any treatment (demonstrator) and the second animal is the recipient of information and is the one that underwent surgical procedures (observer). For the first three days, mice are food restricted to enhance their exploratory behavior and the observer is habituated to the apparatus with doors open and odorant cues present. The odorant cues we used were lemongrass and orchidea smells. On the third day of habituation, we measured which side of the apparatus is preferred by the observer mouse. On the fourth (experimental) day, we used the opposite side chamber as the target chamber, namely we tried to alternate the preference from the preferred side to the non-preferred side through social transmission of information about localization of food. To that end, we fed the demonstrator mouse in the non-preferred side of the experimental cage.

Our results revealed equal exploration of all three chambers of the experimental cage by the observer mouse on the first habituation day (Fig. 2 B1 and B2), while on the third day of habituation there was a strong increase in preference towards the side chamber containing the lemongrass odour (Fig. 2 C1 and C2). That means that orchidea chamber was used as the target chamber on the experimental day. On that day, the demonstrator mouse was fed in the target chamber (orchidea smell) for 10 minutes with the doors closed, and then transferred to explore the other side chamber as well. Afterwards, we put both the demonstrator and the observer mice in the middle chamber and allowed them to interact for 10 minutes. On the final phase, the observer mouse was free to explore for 10 minutes the entire apparatus with doors open. During this phase we optogenetically inhibited HPC to AON projection for the initial 5 minutes of the phase (Laser ON), while for the remaining 5 minutes we did not perform any optogenetic inhibition (Laser OFF). We measured the percent of preference of the target chamber:

[(Time of exploration of target chamber/ (Time of exploration of target + opposite chamber)]*100%.

Our results for the final phase revealed no differences in distance travelled (Fig. 3 A4 and B4) and mean velocity (Fig. 3 A5 and B5) of the observer mouse between the Laser ON and Laser OFF phases. On the other hand, we observed that during the Laser ON phase, the observer mouse retained its preference for the lemongrass chamber (Fig. 3 A1, A2 and A3), as during the day 3 of habituation, while during the Laser OFF phase, there was an increase in the time of exploration of the target chamber (orchidea) (Fig. 3 B1, B2 and B3). These results indicate a potential involvement of HPC to lAON projections in socially-transferred memory recall after interaction with the fed conspecific. However, more mice are required to validate this hypothesis.

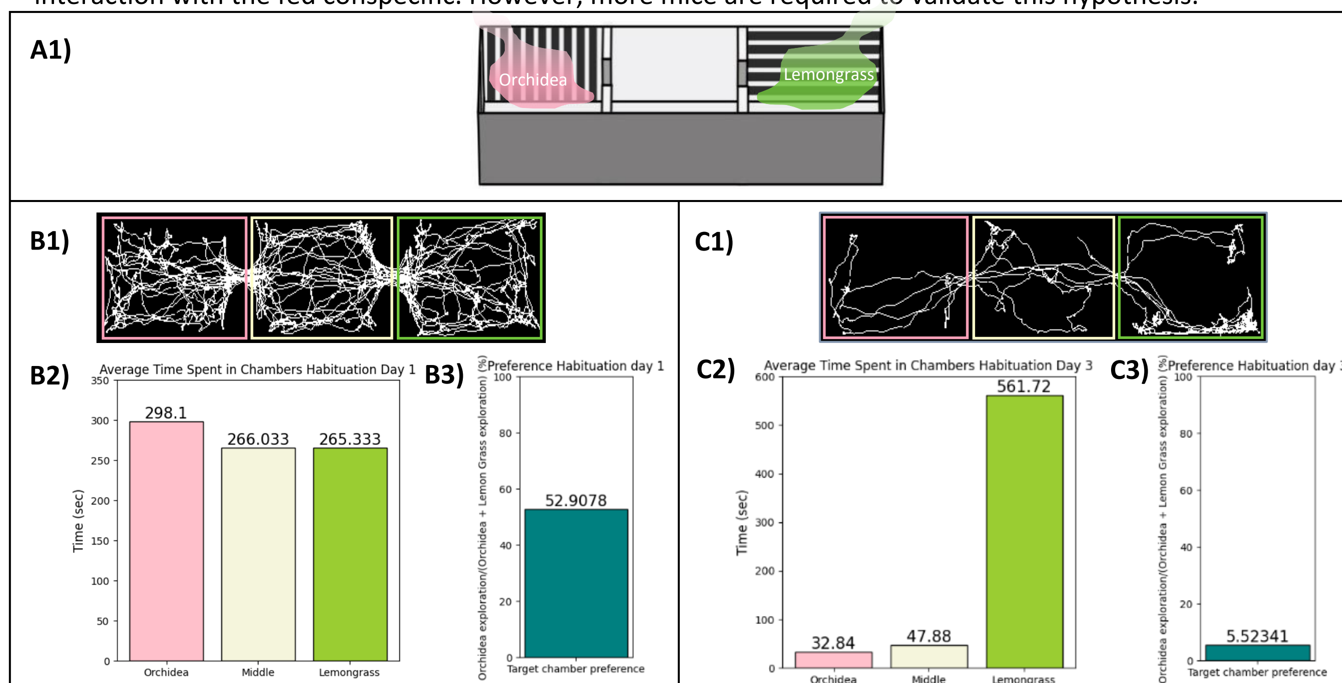

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Figure 2. A1) Three-chamber apparatus with visual and odorant cues as implemented in our experiment. **B1)** Tracking of the animal for 10 minutes in the three-chamber apparatus, **B2)** average time spent in three chambers of the apparatus, **B3)** Preference for the target cage (containing orchidea odor) calculated as explained in the text (day 1 of habituation), **C1)** Tracking of the animal for 10 minutes in the three-chamber apparatus, **C2)** average time spent in three chambers of the apparatus, **C3)** preference for the target cage (orchidea odor) calculated as explained in the text (day 3 of habituation).

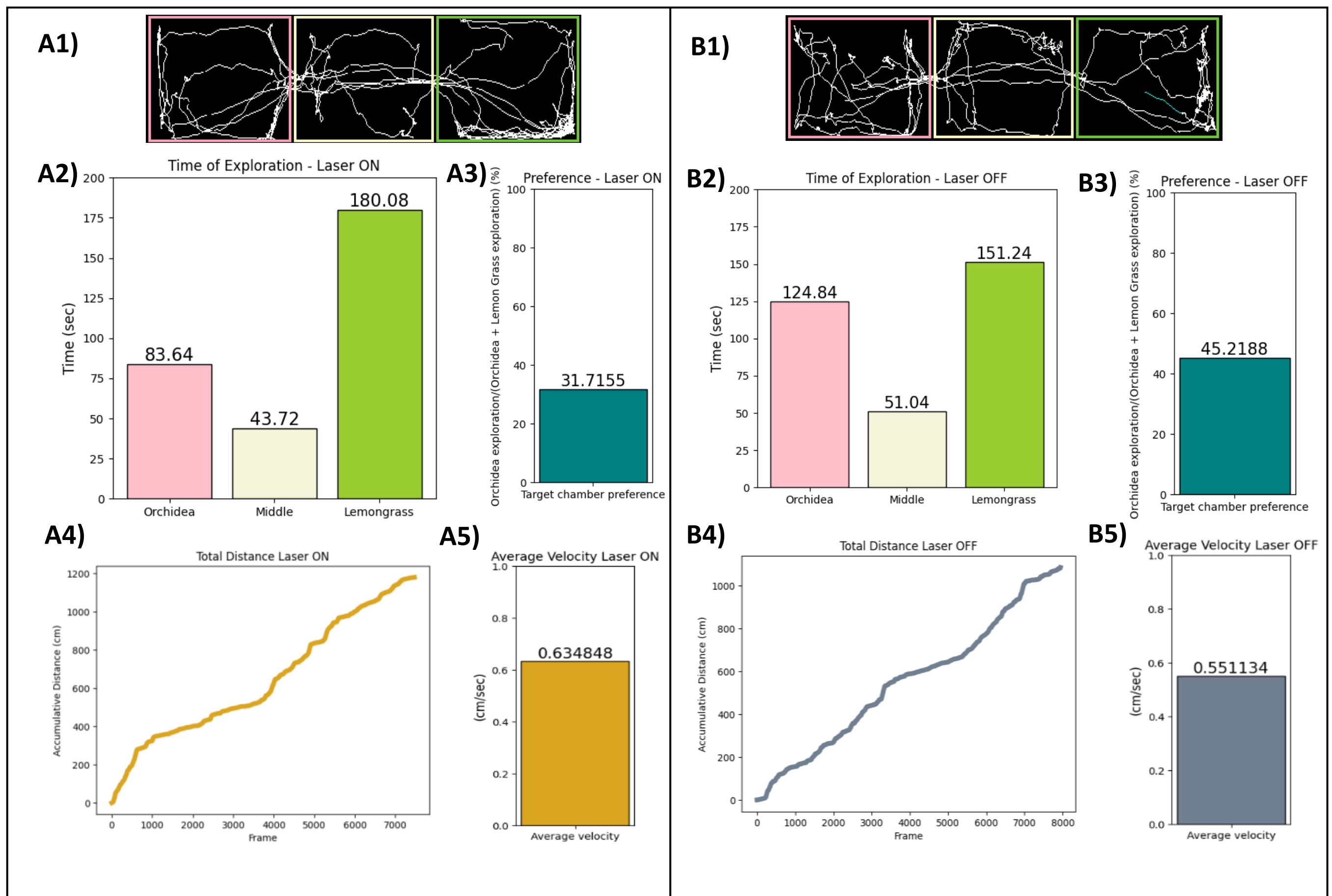

Figure 3. The experimental day, during the laser ON period (initial 5 minutes). **A1)** Tracking of the animal in the three different chambers, **A2)** average time (s) spent in three chambers, **A3)** preference for the target cage (orchidea odor) calculated as explained in the text, **A4)** total distance travelled (cm) and **A5)** average velocity (cm/sec). The laser OFF period (remaining 5 minutes). **B1)** Tracking of the animal in the three different chambers, **B2)** average time (s) spent in three chambers, **B3)** preference for the target cage (orchidea odor) calculated as explained in the text, **B4)** total distance travelled (cm) and **B5)** average velocity (cm/sec).

In addition to the project about the role of the HPC-lAON circuit in social transmission of information about distant food location, during my internship I was also able to attend the course Bonsai Visual Reactive programming provided by the CAJAL Advanced Neuroscience Training Programme and Nencki Open Lab, which took place in Warsaw, Poland on 6-10 February 2023. After this course, among other things, I was able to operate external devices (such as an arduino boards), track animals and monitor their activity, create behavioral tests, such as go-no go tasks with the use of Bonvision and many other features. In fact, using Bonsai RX, I was able to analyze the behavioral experiments I performed during my internship, but also I was able to implement this knowledge for experiments we conduct in my home lab. Furthermore, I also attended

an arduino workshop organized by the Nencki Open Lab and this enabled me to be able to write my own codes for controlling arduino boards, such as control the laser we used for our optogenetic experiments, but also servo motors and sensors.

Additionally, I had the opportunity to participate in stereotaxic surgeries conducted by members of the lab, involving infusion of viruses for chemogenetic experiments in the prefrontal cortex, the brain region I am mainly investigating in my Ph.D. project, but also lens implantation in the hippocampus for miniscope recording. Moreover, I had the opportunity to use and get accustomed with state-of-the-art computational tools that are used in the lab of Prof. Knapska, such as DeepLabCut, a cutting-edge animal pose estimation algorithm. I was trained in how to properly install DeepLabCut, extract frames for labelling of animal body parts, properly train a deep neural network model for pose estimation, but also some troubleshooting. Furthermore, I had the chance to present my Ph.D. proposal and my results heretofore in a lab meeting, during which I received substantial feedback and insightful ideas from the members of the Knapska Lab.

At this point, it should be noted that my internship in the laboratory of Prof. Knapska equipped me with expertise in optogenetics and use of advanced computational tools in neuroscience, which I will be able to set-up in my home laboratory of Prof. Sidiropoulou. This newly acquired knowledge will be invaluable for my Ph.D. thesis in which I aim to delineate adaptations in prefrontal cortical networks of a schizophrenia mouse model, but also it will be utilized for other projects that are carried out in my home lab.

In conclusion, **NENS Exchange Grant** gave me an opportunity to get acquainted with state-of-the-art techniques, work in a new environment abroad for the course of three months, meet and work with excellent researchers and wonderful people from all around the world with different backgrounds and expertise to share, and importantly make good friends. Overall, I characterize my experience as the definition of "broaden my horizons". My training period contributed not only to my scientific competence, but also to my personal development and growth and I am really grateful for the support I received. I am also really thankful for all the members of the laboratory of Emotions Neurobiology for hosting me these three months and making me feel welcome. I would also like to express my gratefulness in particular to Prof. Ewelina Knapska for accepting me to conduct my internship in her laboratory, but also Dr. Ksenia Meyza, Mateusz Kostecki and Konrad Danielewski for all of their help and guidance in all the procedures carried out.

Goodbye party with some of the members of the laboratory of Emotions Neurobiology of Prof. Ewelina Knapska

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