BOCKSTALLER Marie

Joint Master in Neuroscience M1 2011-2012

Summer Project Report

Odour-intensity learning in fruit flies

Supervisor : Ayse Yarali

Institution : Max Planck Institut for Neurobiology, Martinsried

Research Group : Behavioral Genetics (Hiromu Tanimoto)

1. General organization of the lab

a) Max Planck Institut for Neurobiology of Martinsried

During my internship, I worked for a Behavioral Genetics research group. This independent research group is part of the Max Planck Institut for Neurobiology situated in Martinsried, at the southwest border of Munich (Germany). The Max planck Society is an independent non-governmental association of German research institutes. The Max Planck Insitut for Neurobiology is dedicating its research to basic research and investigates the basic functions, structure and development of the brain and the nervous system. This institut is organized as represented in the Figure 1.

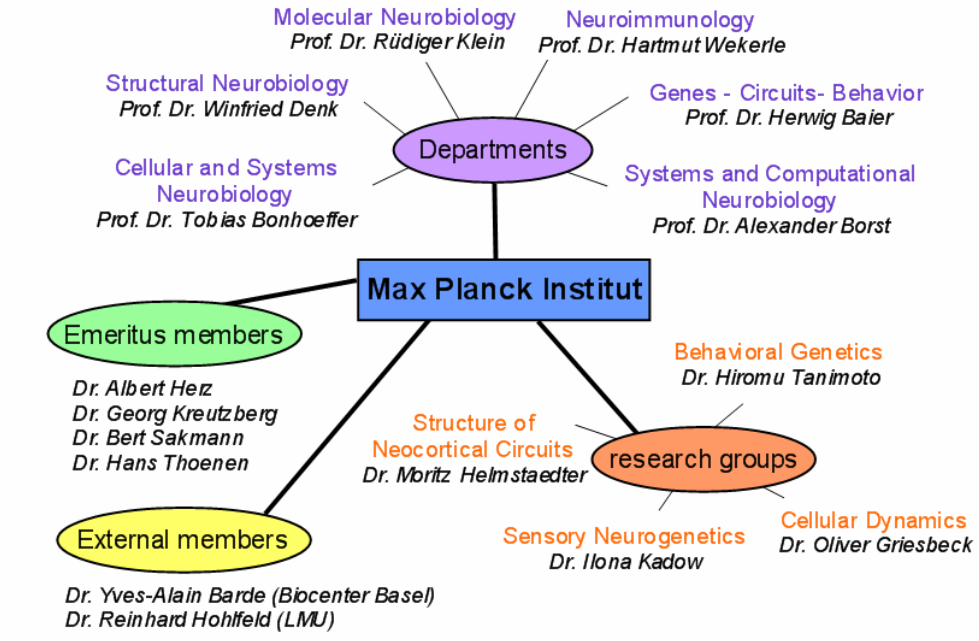


Figure 1. Organisation of the Max Planck Institut (MPI) of Munich. The MPI is organized in 6 departments, 4 independent research groups, Emeritus and external members. This separation in groups and departements doesn't hinder the groups to cooperate on diverse projects

b) Reseach Group : Behavioral Genetics

Since 2008, the group is headed by Dr. Hiromu Tanimoto. The group is composed of 3 post-docs (Dr. Ayse Yarali, Stephan Knapek, Nobuhiro Yamagata) ; 6 PhD students (Mirjam Appel, Dana Galili, Toshiharu Ichinose, Christopher Schnaitmann, Vladimiros Thoma, Katrin Vogt) ; 1 undergraduate student (Oguz Tuba), and 1 staff scientist (Anja Beatrice Friedrich). The different sub-projects the group is currently working on are as following :

1. Elucidating the anatomy and the function of neural circuits underlying olfactory learning with a special emphasis on different reinforcement systems;
2. Mechanisms regulating the expression of associative memories through internal motivational states;
3. Identification of the molecules at synapses executing associative plasticity.

During my stay, I worked with Dr. Ayse Yarali who is working on the first sub-project. Her main topic concerns intensity olfactory learning and the neural structures involved in the distinction between intensities of the same odour.

2. Presentation of my work.

a) The learning paradigm

The experimental part of my internship consisted in performing behavioral tests to investigate odour-intensity learning in fruit-flies. To test for intensity learning, flies are trained with a MEDIUM odour intensity and electric shock. Afterwards, they are tested for their avoidance of either this MEDIUM intensity, or a HIGH intensity. If HIGH induced less conditioned avoidance than MEDIUM, this will mean intensity learning. If HIGH induced as much (or more) conditioned avoidance than MEDIUM, I concluded lack of intensity learning[1].

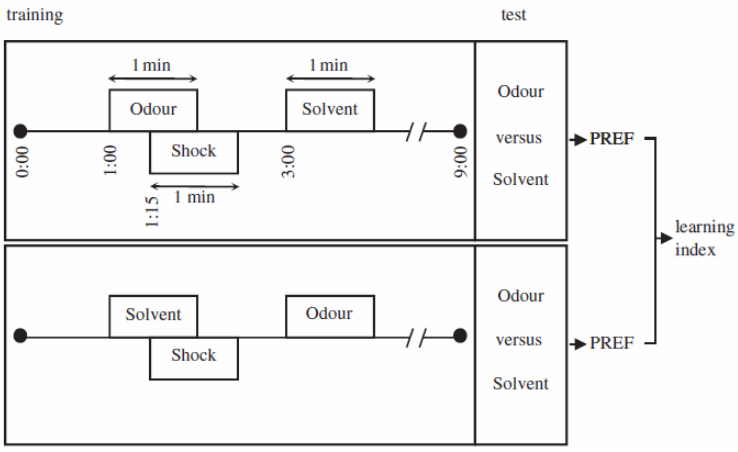


Figure 2. The learning paradigm[1]. Two groups are needed : one trained with the odour associated to shock and a solvent presented alone ; and the second group trained with solvent/shock and the odour presented alone. Each group was then tested for choice between the odour and solvent in a T-maze. Odour preference (PREF) was calculated based on the distribution of the flies (males and females are counted separately). The difference between the PREF values of the reciprocally trained groups then gives the learning index. Negative learning indices demonstrate conditioned avoidance of the odour.

b) Fly crossing schemes : theory of drosophila genetics

One theoritical part of my internship consisted in the understanding of the synthesis of specific genotypes, particularly by using the Gal4-UAS system[2]. This system was used in the lab to block multiglomerular projections neurons (mPNs) : the hypothesis was that those neurons could be implicated in coding odour-intensity*. To generate flies lacking these cell populations, you first need one kind of transgenic fly, in which the Gal4 transcription factor will be expressed in the neurons of interest under the control of an appropriate promoter/ enhancer. In a second kind of transgenic fly, the expression of tetanus toxin light chain (TNT) will be under the control of the upstream activating sequence (UAS). In the progeny of these two kinds of fly, the Gal4 expressed in the desired mPNs (GH146-Gal4) will bind to the UAS, inducing the expression of TNT, leading to an inhibition of neurotransmission and blockage of exocytosis in these neurons. Nevertheless, note that the GH146-Gal4 driver line also targets uniglomerular projection neurons (uPNs) and other neuronal populations that are not hypothetized to be involved in intensity learning but could interfere with the intensity learning process (see section 3.). Future researchs will focus on targeting mPNs more specifically.

---

[1] Yarali, A., Ehser, S., Hapil, F.Z., Huang, J. & Gerber, B. 2009 Odour intensity learning in fruit flies. Proc. R. Soc. B 276, 3413–3420

[2] Brand AH, Perrimon N. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development, 118, 401–415.

* the two scenarios are described in section *3.*

c) Fly husbandry

I learned how to perform basic fly husbandry. Actually, fly husbandry is used to get successive crosses and to finally reach the genotype of interest. From the successive crosses, you need to collect either virgin females or males (Fig. 3). Depending on the balancer present at the level of one or more of the four chromosomes of the fly, you can get specific markers (curly wings, hairs, colour of the eyes) (Fig. 3) . To collect flies, they are first anesthetized under CO^2, selected using the markers, and put into a new vial. You then need to let the population grow : flies are kept at 25°C with 60% of humidity in food vials, that need to be changed every two days by using the *flipping technique***. You keep the old vials at 25°C (60% humidity) in which larvaes will grow and give rise to the next fly generation within 10 days.

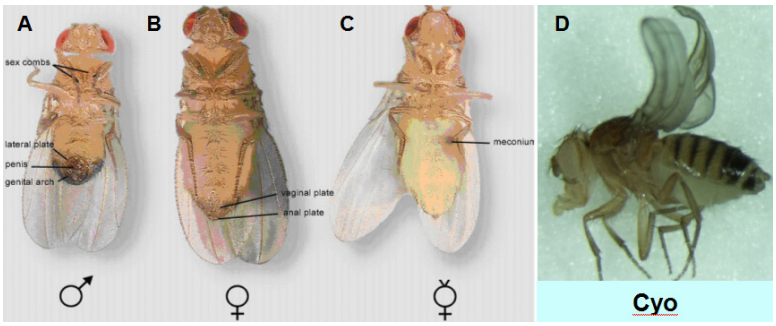


Figure 3. Fly markers. A) Sex markers of the male are sex combs on the first set of legs and a pair of brown claspers on the abdomen B) In female, sex combs are absent but they have a brown, hairy abdominal. C) virgin females have a pale pigmentation and a dark spot in the translucent abdoment. D) By using balancer chromosomes in your crossing schemes you could easily select your flies : balancer chromosome leads to a specific phenotype like here the curly wings typical for Cyo balancer of the second chromosome. Balancer also block further recombination on the homologous chromosome, meaning that once you cross a fly with the mutation of interest with another fly carrying the balancer of the same chromosome, you make the mutation stable for the next generations.

d) The computational model

Dr. Ayse Yarali worked in collaboration with Prof. Andreas Herz (LMU, München) to build a computational model of odour-intensity learning in drosophila. I joined the weekly meetings where they tried to adjust the model. I read the first project description[3] about the elaboration of the model. Briefly, the distribution of the firing of the olfactory receptor neuron (ORN) in response to the different concentrations of one odour follows a sigmoid curve. Thus, they want to create a model containing all the possible sigmoid curves for the different odors concentrations and ORNs. They setted different parameters for the curves : the concentration at the turning point of the curve (*a*), the slope (*b*), the maximum firing rate (*fmax*). To select appropriate parameters they are comparing their findings to the ones of a previous paper, where the response profile of the 24 ORNs to 100 different odors was estimated[4]. The final model should be able to predict the response of any ORN to any concentration of any odour. The work between the two teams is splitted such that the team of Dr. Yarali provides the behavioral datas that will help to build and to test the model, whereas the team of Prof. Herz is programming the computational model itself.

** You tap the flies down to the bottom of the old vial, you quickly remove the vial's plug, you place an open fresh vial down on top of it, holding the two vial mouths together, you flip them over and tap the flies down into the new vial.

[3] Yarali, A., Nehrkorn, J., Tanimoto, R. & Herz, A.V.M. 2012. Modeling Olfactory Receptor Neuron responses in Drosophila. [not published yet]

[4] Halem, E.A & Carlson, J.R. 2006. Coding of odors by a receptor repertoire. Cell 125, 143-160.

3. **Integration of the work in the global picture**.

Animals use olfaction for detecting food, predators or mates, whereby they rely on both odour-quality and -intensity. Although neuronal coding of odour-quality is fairly well studied, it remains unclear how odour-intensity is coded. Actually, it has been shown that flies discriminate between odour-intensities: Having experienced a medium intensity of an odour together with shock, flies later on strongly avoid this medium intensity, but not lower or higher intensities, reflecting intensity-specific learning[56]. I could reproduce this result : the flies strongly avoided the medium intensity but the avoidance was decreased when an higher intensity was presented (Fig. 4). This difference was significant ($p<0.025$, Mann Whitney Test, Statistica).

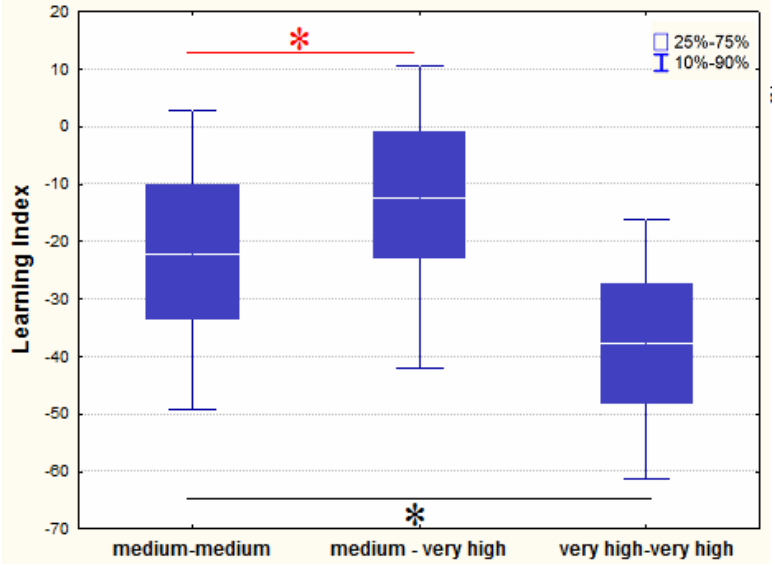
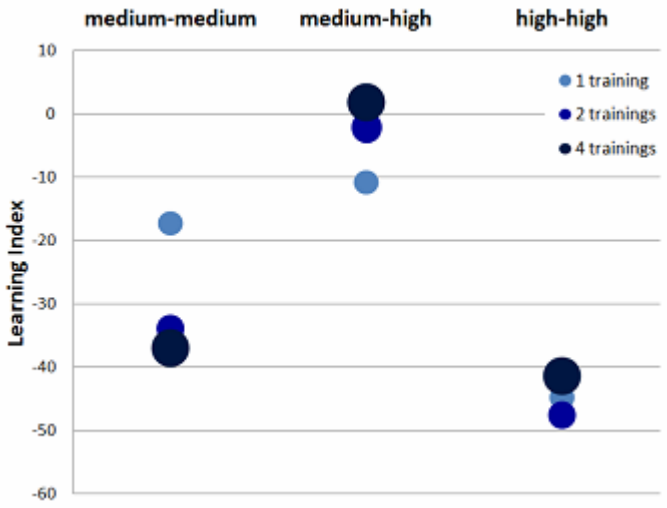


Figure 4. Odour intensity Learning. The results were obtained following the learning paradigm presented in a). For the medium and very high intensity, 10^{-4} and 10^{-2} methylcyclohexanol (MCH) dilutions were respectively used. The flies were either trained and tested with the same intensity (medium-medium or very high-very high) or with a different intensity (medium-very high). Note, that when the same intensity is presented during training and test, the learning index increases with the intensity. The decrease in performance in the condition medium-very high compared to medium-medium is consistent with the intensity learning hypothesis. Flies are not avoiding the odour as much as for the condition medium-medium, because they also encoded the intensity of the odour during training and can distinghish this intensity from the one presented during test. * $p< 0.025$



Then, I wanted to characterize intensity learning in more details. To achieve this, I decided to test whereas increasing the number of trainings could affect the performance of the flies. Already with two trainings, the flies avoided the medium intensity significantly more and the avoidance was also significantly decreased when an higher intensity was presented compared to the avoidance indices obtained with one training (Fig. 5).

Figure 5. Repetition increases intensity specificity of memory. By increasing the number of trainings, we could improve the performance of the flies. The learning index, when presenting the same MCH medium intensity during training und test, was significantly increased with two and four trainings compared to one training ($p< 0.025$). Odour intensity learning was also better, since the learning index was significantly lower in the condition medium-high with two and four trainings compared to one training.

[5] Yarali, A., Ehser, S., Hapil, F.Z., Huang, J. & Gerber, B. 2009 Odour intensity learning in fruit flies. Proc. R. Soc. B 276, 3413–3420.

[6] Yarali A, Gerber B 2010. A neurogenetic dissociation between punishment-, reward- and relief learning in Drosophila. FrontBehavNeurosci 4: 189.

One question remains : how does it neuronally work ? Odours activate olfactory sensory neurons (OSNs) in the fly antenna. OSNs with the same odour-responsiveness converge onto a glomerulus in the antennal lobe, where uPNs pick up the information. uPNs project to the pre-motor lateral horn (LH) and to the mushroom bodies. But what about intensity-coding along this olfactory pathway? Typically, the OSN- or uPN-activity patterns induced by a medium odour-intensity remains nested within the pattern induced by a higher intensity of the same odour[7]. Any memory trace established at these levels by a medium odour-intensity would thus be fully read out with a higher intensity, contrary to the behavioural observation[3]. At the next level, at least some MB-cells seem intensity-specific, making intensity-learning conceivable[8]. In this scenario, odour-quality and –intensity memories would both be laid down in the MB-cells. An alternative scenario must be considered: Odour-quality and –intensity memories may be segregated. Some atypical PNs in the antennal lobe receive input from nearly all glomeruli, thus "calculating" the total antennal lobe activity[9]. These mPNs would "know" how much odour is there, without "caring for" which odour it is. During my summer project, I focused specifically on this second hypothesis and elaborated a crossing scheme, where mPNs were blocked (see section c)). Using our learning paradigm, we could show that in the genotype of interest (GH146-Gal4 ; UAS-TNT) no learning was observed, when the same odour intensity was presented during training and test (Fig. 6). Thus, we decided not to test the condition medium-high, since no decrease of the learning could be potentially observed. We conclude, that olfactory learning (composed of odour-quality and –intensity) is lost when GH146 projection neurons are blocked. Further researchs could help us to determine more closely the neuronal population exclusively involved in odour-intensity learning.

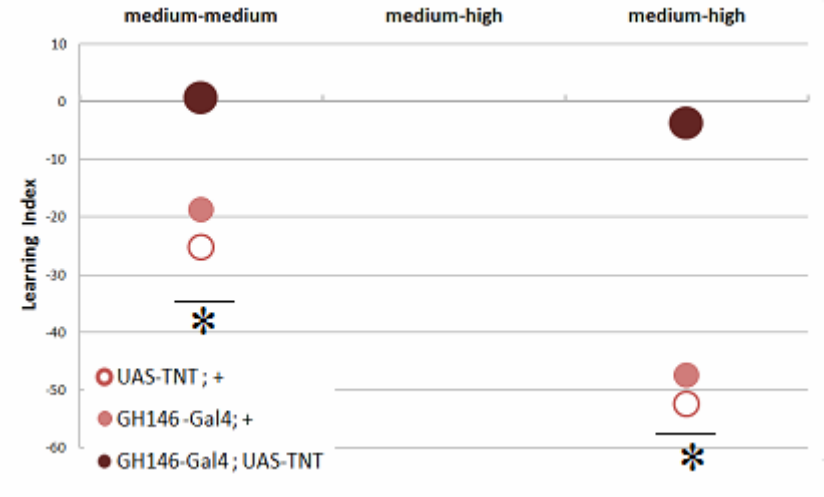


Figure 6. Olfactory learning is lost in flies where mPNs are blocked. Learning indices of GH146-Gal4 ; UAS-TNT flies are not significantly different from 0 but differ significantly (*$p < 0.025$) from the indices of control groups. The lack of avoidance could be due to a perturbation of the learning ability or of the smelling faculty. Further researchs are needed to answer this question. We conclude that a set of the neurons blocked in this line could be involved more specifically in intensity learning.

[7] Ng, M., Roorda, R. D., Lima, S. Q., Zemelman, B. V., Morcillo, P. & Miesenboeck, G. 2002 Transmission of olfactory information between three populations of neurons in the antennal lobe of the fly. Neuron 36, 463–474.
[8] Stopfer, M., Jayaraman, V., Laurent, G. 2003. Intensity versus Identity Coding in an Olfactory System. Neuron, 39, 991–1004.
[9] Marin, E. C.; Jefferis, G. S.; Komiyama, T.; Zhu, H. & Luo, L. 2002. Representation of the Glomerular Olfactory Map in the Drosophila Brain. Cell, 109, 243-255.

Altogether, the behavioral experiments I performed confirmed the existence of odour-intensity learning and my collaboration to the elaboration of the fly-schemes and crosses will help to confront the two proposed hypotheses of how intensity learning neuronally works.

4. Personal feelings

During this internship, what I liked most was performing the behavioral experiments. It was also really nice to be integrated into the design of the crossing schemes : I could learn how with a limited amount of crosses you can create the genotype you need. I also could learn about the specific markers used to recognize the genotypes.

Actually, this was also the most challenging part : I had to learn, that the crossing-schemes you elaborate do not always work as you expect. This counterpart can delay an experiment for months, since you need approximately 10 days to get the first offspring, and many successive offsprings to get the genotype you are looking for... Moreover, behavioral testings are not as easy as it seems : even by performing exactly the same experiments, it happens that the results vary. This means that accuracy of the manipulation, timing, attention are really critical when performing behavioral testings : at the end, you want to be 100% sure that the differences observed are really due to the genotype of the flies and not due to mistakes or biases you introduced during the test.

During those three months, my aim was to develop new practical skills, and to become independent performing behavioral tests on flies. This goal was achieved, and I could obtain results further contributing to the understanding of how odour-intensity learning works. Such a specific training was not possible at my home university, since these techniques and model organism are not used in the laboratories affiliated to the Joint Master of Neuroscience. Furthermore, I really appreciated to be allowed to work pretty independently, importantly though, I was not “left alone”. I had plenty of interaction with my supervisors, was directed to the critical literature and attended interesting talks. Finally, my supervisors offered me the great opportunity to present my work and the whole odour-intensity learning project at the honeybee-drosophila meeting of Constance at the end of September. Confronting my project to scientists specialized in this field of research was a great experience and was a significant step forward in my scientific training.