Who's a good bee?

Francesco Bondini[1], Barnabás Béres[2], Melita Dubravcic[3], Ester Zita Szatmari[4], Anastasija Vasilijevic[5]
Mentors: Alexandre Kempf[6], Eduarda Susin[6]

[1] Calabrese – Levi, San Pietro in Cariano (VR), Italy
[2] ELTE Radnóti Miklós Gyakorló Gimnázium, Budapest
[3] V. Gymnasium, Zagreb, Croatia
[4] Székesfehérvári Vasvári Pál Gimnázium, Budapest
[5] Gimnazija u Čačku, Čačak, Serbia
[6] CNRS, Paris, France

Motivation

Both humans and animals can learn. In this project, we used bees to help us understand the process of learning and it's affects on their brain, which can be implied on the human brain. The important role in learning in the bee brain have octopamine, which is a neurotransmitter and neuron VUMmx1. VUMmx1 collects infomation from antennal lobe, lip region and lateral protocerebral lobes. It responds only to sugar, and is responsible for olfactory learning - associating the specific odor with the reward.

Classical conditioning (Pavlov experiment)

Classical conditioning is form of learning which involves behaviour learning through association.
It was described by russian physiologist Ivan Petrovič Pavlov (1849-1936). Pavlov experiment included dogs. For few seconds before Pavlov would give the dogs food, the bell was ringing, and it rang while the dogs were eating. That was repeated until dogs started to salivate on the sound of the bell,even when there is no food. Pavlov named the food the uncoditioned stimulus, because it naturally provokes the salivating, and he named the salivating uncoditined response.
Bell sound is conditioned stimulus, because it provokes salivating only when there is food. Salivating on the bell sound is conditioned response. Condtitioned response is reaction learned through conditioning. Conditioning is the process of pairing the uncoditioned stimulus with the conditioned, in order to get the uncoditioned response for the conditioned stimulus.
In our project, we used this experiment on bees.

The unconditioned (biologically relevant, reward) stimulus is paired with a conditioned (neutral) stimulus in order to get the same reaction for a conditioned stimulus.
In our project, we used classical conditioning to teach bees distinguish odors.
As uncoditioned stimulus we used sugar and the uncoditioned response (reflex) was proboscis extension. The conditioned stimulus was the odor A (lemon).
By repeating the experiment, bees learnt to extend the proboscis only for odor A, so the uncoditioned response became the conditioned one.

Experiment

The experiment was repeated throughout 3 days, using 20 bees for 15±3 trials per repetition. The bees (*Apis mellifera*) were caught at Davorin Krakar's bee garden in the summer 2018 morning hours in Požega, Croatia. They were caught by 5 individuals with falcon tubes and then immediately put to sleep in a cold environment. After a few minutes, the bees were taken out of the cold and carefully positioned into eppendorf tubes with tweezers. While still sleeping, they were taped into position using scotch tape, scotch tape being placed between their head and thorax. Bees taped in eppendorf tubes were then organised in a holder made out of styrofoam so they could be easily identified by their ordinal number (picture 1).

Picture 1. Bees in a styrofoam holder

During the experiment bees were introduced to odor A (lemon) and odor B (blueberry) for 3 seconds per every trial. The odors were prepared in a seperate room from the one that the bees were in, so they couldn't sense them before the start of the experiment.
For preparing the odor A (S+) we squeezed a lemon fruit (*Citrus limon L.*) and used its juice to soak a piece of paper which we proceeded to put in a syringe. We drained all the liquid out so that when the syringe was pressed the flow of scented air came out.
For preparing the odor B (S-) we did the similar thing, but instead of using blueberry juice, we used ethyl butyrate to soak the paper with.
The odor which will be used in a following trial was determined by a pseudo random pattern (S+, S+, S-, S+, S-, S-, S+, S-, S+, S+…).

When a bee is introduced to odor A (S+), she had 3 seconds to lick, if she didn't lick, her choice was marked as incorrect. In that case, for the next 2 seconds her antennas were touched with a toothpick coated with sugar so she extends her proboscis (proboscis extension reflex) and realises there's a reward (sugar) in front of her. If a bee licked without us having to touch her antenna, her choice was marked as correct, but If she licked after we touched her antenna or didn't lick at all, her choice was marked as incorrect.

When a bee is introduced to odor B (S-), she had 3 seconds to extend her proboscis, if she didn't lick, her choice was marked as correct and if she licked her choice was marked as incorrect. No antennas were touched while introducing bees to odor B (S-) since bees shouldn't associate odor B with a reward. There was no reward given to the bee for odor B.

Experimental data was recorded in MS Excel tables and later analysed and plotted using Python 2.0.

The Learning rule

We used a mathematical model to make a simulation to examine the process of learning. We can describe brain as groups of neurons. Every group is responsible for something, in our case *y* represents the group, which is responsible for making the bee to decide to lick or not to lick.

$$y = \theta(\vec{\omega} \cdot \vec{x} - \xi)$$

θ is a heaviside function, we need this because it is a two-state system, it must be 1 or 0 (1 means if the bee licks, 0 if she does not do it). We expect *ωx* to be a rational number between -1 and 1. As every system makes mistakes, so the brain of the bee. *ξ* represents this mistake, it is a rational number between -1 and 1, which we generated randomly.

$$\Delta \vec{\omega} = \alpha f(R - \vec{\omega} \cdot \vec{x}) \vec{x}\, y$$

We are capable of calculating the synaptic weights. The synaptic weights show us the quality of the connections between the different groups of neurons. α is the learning rate, it stands for the speed of learning, usually smaller than 1. *f* is an asymmetrical function, bees learn much faster if they get reward. This means, they learn it faster to lick when there is sugar water than not to lick when there is not. *R* is the presence of the reward, it is 1 if it is there and -1 if it is not. *xy* shows which coordinate changed. If *y* is 0, so the bee did not lick there was no learning, nothing happened, otherwise *y* is 1. The coordinates of *x* can only be 1 or 0. It shows if the odor was present and if the bee was correct or not.

$$\begin{bmatrix} 1 \\ 0 \\ 1 \end{bmatrix} \begin{bmatrix} 1 \\ 1 \\ 0 \end{bmatrix}$$

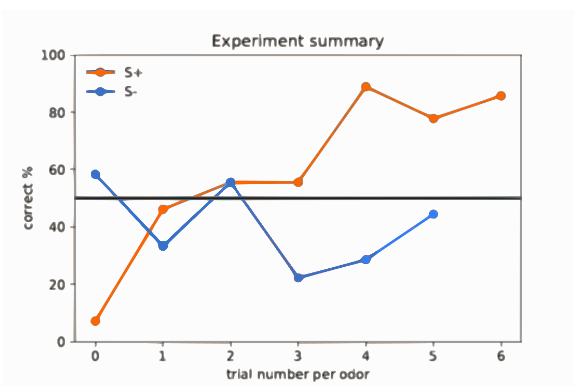
Possible values of *x*

Results

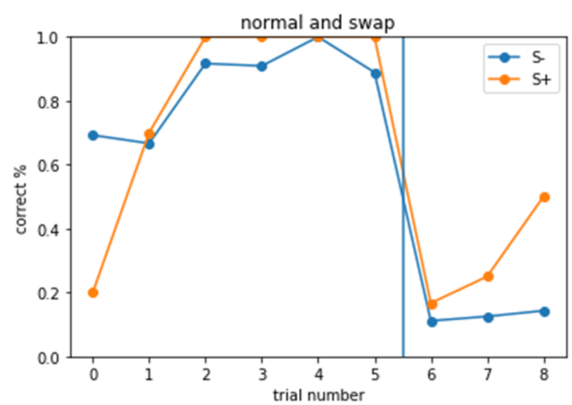
In the first experiment, with 20 bees, the S+ odor was made of ¾ lemon odor and ¼ blueberry odor while the S- odor was the opposite (¾ blueberry and ¼ lemon). The blueberry odor was stronger than the lemon one and so it was quite difficult for the bees to discern the two different odors and lick with the right one. In addition to that the bees were really thirsty and exhausted because of the hot day when we've done the experiment (fig 1).

In the second experiment (14 bees) we used 100% concentrated odors (S+ lemon and S- blueberry) and it was better than the day before, in fact the learning curve is excellent (fig 2 until the 5th trial). In the second part of the experiment we switched the reward odor, so the bee received the reward with the S- odor and didn't with the S+. As you can see in the second figure after the 5th trial the correct answers percentage fell down and the learning process was slower. Unfortunately we don't have the full learning curve of the second part because the bees where exhausted and half of them died during that part of the experiment.

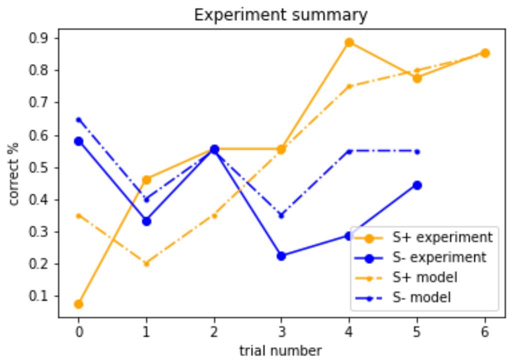
By using the model of learning we made a program to simulate the learning curves of bees and compare them with the real ones (fig 3). Firstly we changed the values of the constants until we obtained curves that were quite similar. Some of the datas for the model were chosen randomly like the starting strenght of the synapses because they were variables that change from one bee to another. So we also did 1000 simulations and plot the average and the confidence interval together with the real bee curves (fig 4).



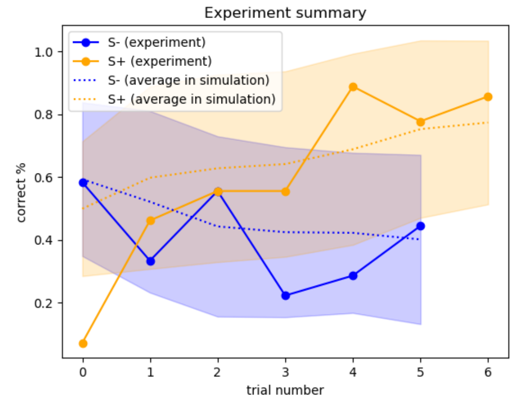
20 bees, 11 total trials (6 trials with S+ and 5 with S-)



14 bees, 18 total trials (9 for each odor).
After the 5th trial: reward switch



Here it is shown the same graph of Fig.1 but in addition to that it is represented also 1 simulation of the model (which is the dotted line)



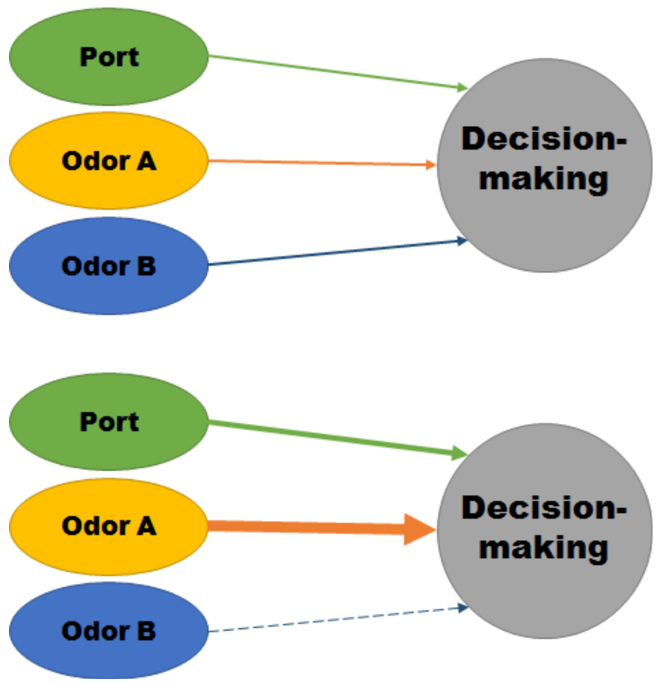
By repeating 1000 simulations we calculated the average and plot it as a dotted line. The colored areas are given by the confidence interval of the simulations

In the y axis it is shown the percentage of correct answers which is an average of all the bees in that trial. Trials are represented on the x axis.

In the graph there are shown 2 different lines which represent the learning of the 2 odors although they were presented randomly to the bees. So each point is the average of correct answers in a specific trial of S+ or S-.

Discussion

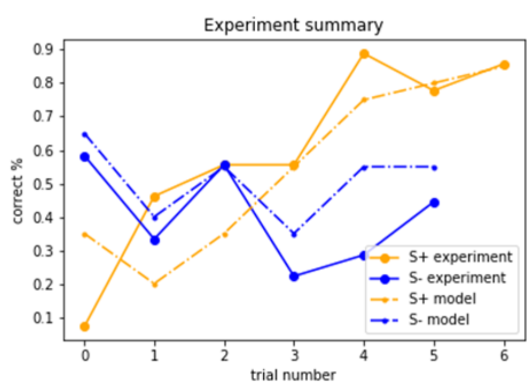
In our case, as we stimulated the olfactory system, the following schematic illustration can be shown: “Odor A” (S+) and “Odor B”(S-) correspond to the group of neurons that are being activated by the stimuli of the presence of the lemon and blueberry odor respectively. “Port” covers all the neurons that can have some side effect on the results, like perceiving of temperature, air movement, etc. This module also includes the neurons shared mutually between “Odor A”(S+) and “Odor B”(S-).



Conspicuous differences can be discovered instantly, when looking at the plotted curves of the data, however, the shape of the curves, therefore the outcomes of the experiences are slightly similar. This phenomenon is due to the Hebbian progress, which takes place in the nervous system while learning. It means, that synaptic plasticity is present in the brain, so when two, connected neurons fire suddenly after each other, their synaptic connection will strengthen due to synaptic potentiation. In contrast, when a firing is not being followed by the connected neuron, the mutual synapse will weaken and synaptic depression occurs.

In our case, the already shown illustration is valid (see explanation at *The Learning Rule*). After learning proceeds, the synaptic weights of the mentioned modules change because of synaptic plasticity.

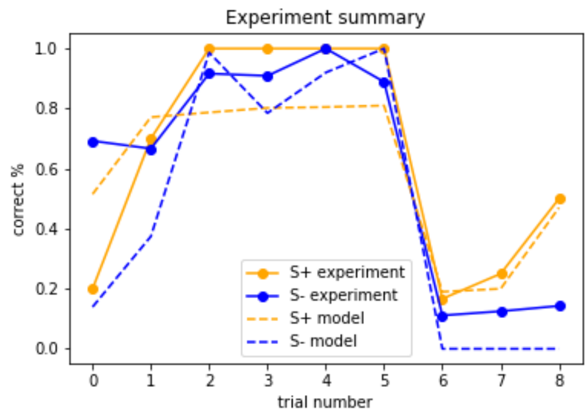
In the first experiment, after 11 trials bees were able to associate S+ with reward almost perfectly, however, learning for S- did not proceed at the same level. The most proper interpretation for this is that the used odors were mixed, in a ratio of 3:1. As ethyl butyrate (blueberry) was more concentrated itself as the squeezed lemon, the former one was too dominant and hardened the odor-distinction task.



Another impediment for more proper results was the number of trials. Because of the limited time we had for the experiments, the learning process of our models could not reach its peak, however an upward trend is present in each cases. This predicts, that if more trials were accomplished, learning would have been fulfilled.

Due to the lack of laboratory-like circumstances, several factors played in role of altering the learning process, and caused the death of bees too. High temperature, air blast and thirst of the

bees were among these factors for instance. Therefore it is important to mention, that while every experiment was started by using 15-20 bees each, at the end of the trials only a small proportion of them survived (3-4 bees). Because of this, data gets more unreliable by time, as the number of source reduced.
Lastly, because of undetected bugs in our Python code for the learning model, it could not function properly in the case of the stimuli-altered experiment. This is the reason why S+ model's curve does not reach the peak, and why S- model curve remains zero always after changing the stimuli.



Aknowledgments
We would like to thank Nikolina Šoštarić and Dora Grbavac as organizers of Summer School of Science S3 camp, and Davorin Krakar, the bee keeper, for allowing us to use his bees for this project.