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Motivation

In the 20th Century Ivan Pavlov Trained Dogs to associate the sound of a buzzer with the presence of food and salivate on command. By measuring salivary secretion as a quantitative measure of the higher nervous activity, Pavlov was able to see how strongly the dog picked up on a new behavior unconsciously by just associating two different stimuli. As more research was conducted into this field of conditioned behavior we have observed that nearly all vertebrates and even some invertebrates are capable of learning to associate a stimuli with a completely different and distinct stimuli.

Problem statement

However it raises the question, does an organism need to have a nervous system to exhibit a non voluntary response to some stimulant such as Pavlov's dog did with their buzzer?

Related work

For this project we Read two papers on conditional behavior in organisms where it has never been observed before, and referenced several more that either support or contradict the

researchers conclusions. The first paper we read was on Conditional behavior in amoeba :

Ildefonso M. De la Fuente et al, “Evidence of conditioned behavior in amoebae”, Nature (2019)

Loy, Ignacio et al. “Where association ends. A review of associative learning in invertebrates, plants and protista, and a reflection on its limits.” *Journal of experimental psychology. Animal learning and cognition* vol. 47,3 (2021): 234-251. doi:10.1037/xan0000306

Followed by a scientific paper that conducted experiments to see if plants could form conditional behaviors along with dissenting opinions on the experiment:

Gagliano, Monica, et al. "Learning by association in plants.", Nature (2016)

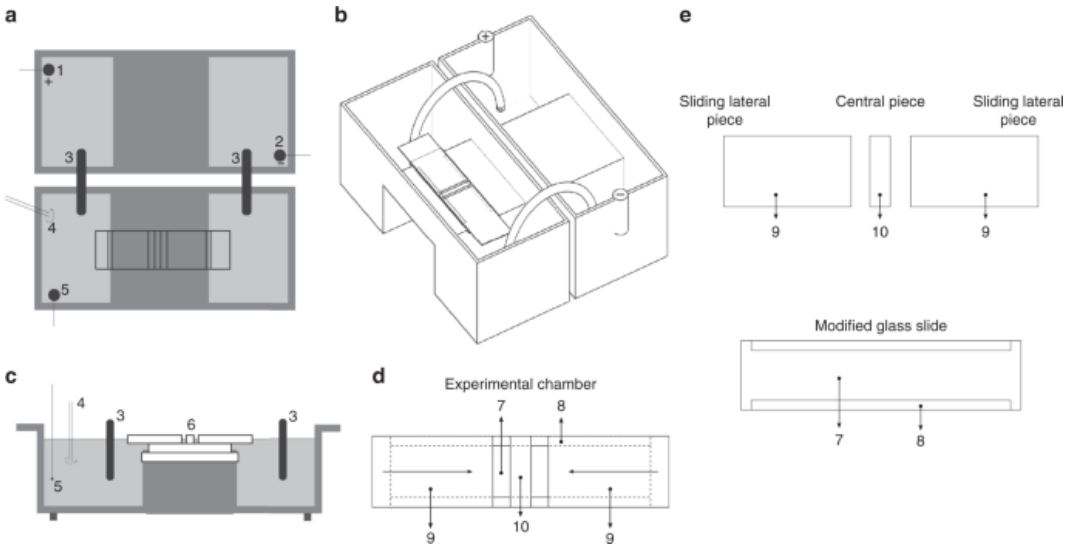
Markel, Kasey. “Lack of evidence for associative learning in pea plants.” *eLife* vol. 9 e57614. 23 Jun. 2020, doi:10.7554/eLife.57614

Mallatt, Jon et al. “Debunking a myth: plant consciousness.” *Protoplasma* vol. 258,3 (2021): 459-476. doi:10.1007/s00709-020-01579-w

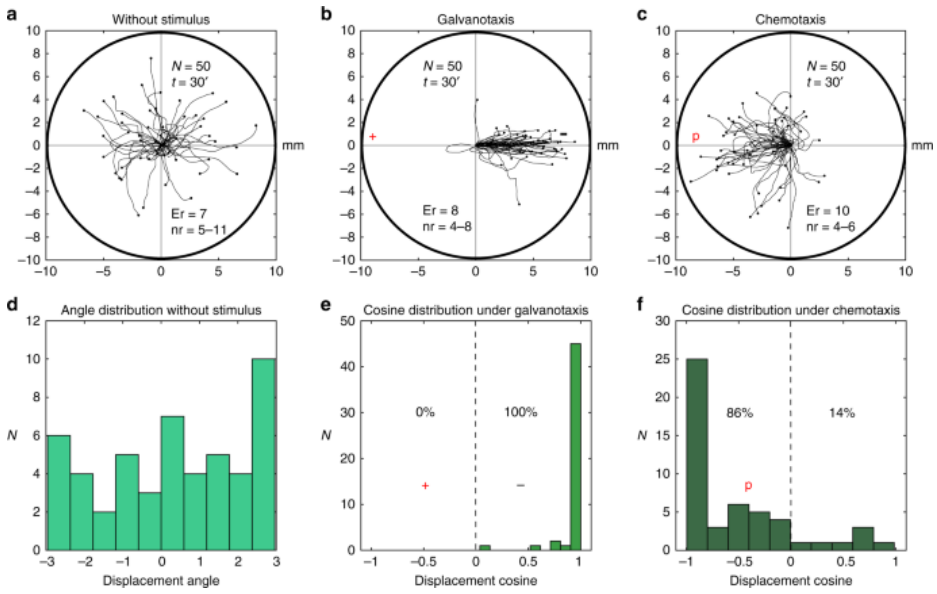
Setup

Conditioning in Amoebae

The researchers used two standard electrophoresis blocks connected via an agar bridge to link the two together, only the back block was connected to power directly, while the front block was the experiment chamber and was only connected via the bridges. Placed in the chamber was a sliding lateral piece of glass which contained the amoeba and was used to store them for placement and removal. Example Illustration:



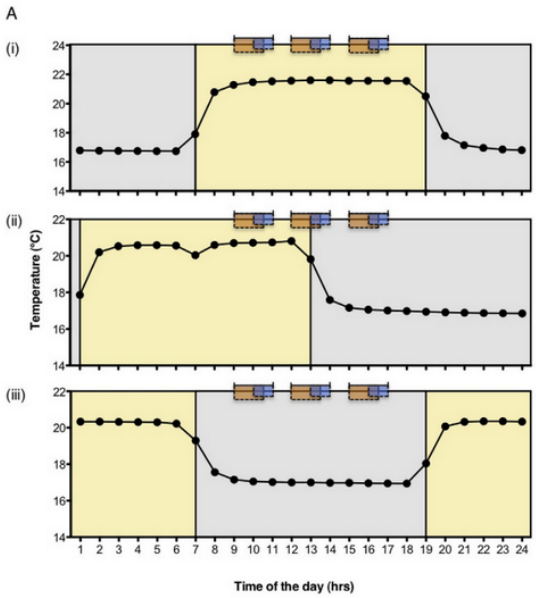
Amoeba proteus, the main amoeba used in this experiment is attracted to a certain peptide, nFMLP, that it uses to track down food it also happened that Amoeba proteus has a behavior where it is attracted to the cathode of an electric field. These were the two stimuli that the researchers were planning on using for conditioning. First baselines were taken of the cells reacting to each stimuli separately, then they performed an induction step where the peptide nFMLP was placed near the anode inorder to condition the amoeba to migrate to the anode instead. The motions of the cells were monitored using a camera attached to a microscope to trace their paths, the directionality of each cell was quantified by the cosine of the displacement angle. Here you can see the results of each stimulus individually before conditioning:



Conditional learning in Plants

The researchers conducted two different experiments to explore a pea seedling's ability to be conditioned into a behavior and how well effective conditioning is. The first experiment was to test if a pea seedling could be taught a behavior at all. They tested this by placing the seedling into a planter where it would be covered with a Y maze. Two different testing groups were to be conditioned with one control group that would receive no conditioning. To achieve such conditioning on the seedlings, the researchers would train the seedlings through the use of wind with a fan and light with a blue light bulb. One test group would receive a gust of wind for an hour where the seedlings would receive 30 minutes of light simultaneously followed by 30 minutes of only light down one end of the Y maze. The second test group would receive the same time durations as the first test group, however instead of the conditioned stimulus, the wind, and unconditional stimulus, the light, being down the same Y pipe end they would be at opposite ends. They would do this training for three days where at the end of the day the plants would remain undisturbed and examine the next day to see which pipe end they grew into.

In the second experiment, it was tested at what time the plants would best learn. Again there would be three groups, one group would receive conditioning just like in the first group in the first experiment but during hours when the sun would usually be up, the second group would be conditioned from midnight until midday, and the third group would receive conditioning only when the sun would usually not be up.



Results

Conditioning in Amoebae

After induction cells appeared to show symptoms conditioning and continued to show signs for up to 90 minutes after the induction was performed. It was noted by the researchers that the time after induction where signs of condition were still noticeable was significant as compared to the life of the organism. When the amoeba were introduced to an electric field after induction a significant amount of them now migrated to anode even when there was no peptide present. In a follow test the amoeba were exposed to the field for 30 minutes and then given a five minute break before the field was switched back on but with the opposite polarity and the amoeba still showed signs of conditioning.

Conditional learning in Plants

In the first experiment, it was shown that the plants were in fact capable of being

conditioned. If the plants were trained with light and wind with one end of the Y maze they would grow towards that end and if they were opposed the plant would grow into the arm opposite of the end where the wind pipe was. Using this information that plants show the capability to learn a conditioned behavior they went on to the second experiment. Their results from the second experiment is that while plants do still have the ability to learn a conditioned behavior, what time of day they are being trained really affects the effectiveness of the training. When the training takes place during hours when the sun is up is when plants best learn where if they are trained when the sun is never up, they do not learn. In essence, they claim that their results show that plants in a sense 'sleep' just like vertebrates.

Conclusions

With two different invertebrates and two vastly different experiments showing that invertebrates are capable of, if not display evidence of learning behaviors through conditioning. While there has been much pushback and dissenters of plants having the ability to learn through classical conditioning, if learning at all, from scientists such as Jon Mallatt who calls plant learning 'a myth' and Kasey Markel who demonstrated by recreating experiments done by Monica Gagliano, the scientist that conducted the plant conditioning experiments, you will not get the same results that the original experiment received; and goes on to claim that there is a lack of evidence that plants have the ability to learn. While much research has to be done to better understand if invertebrates may actually possess the capabilities to be conditioned into behaviors, especially plants, the prospects are quite astounding. If given the opportunity to redo this project and presentation, our first objective would be to trim down on the words within the presentation as well as present other related work to this project such as the experiments that failed to replicate the conditional experiments done for plants. Much of our issues stemmed from

inefficient time management and if we were given the opportunity to, better planning would be in place.