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Molecular circuits for associative learning in single-celled organisms.

Motivation:

The motivation for this study is the desire to discover if associative learning is present in single-celled organisms. This would be a major deal seeing as associative learning has been spotted in various other multicellular species however that raises the question. Is it possible for a single-celled organism to have associative learning?

Problem Statement:

Is it possible for a unicellular organism to provide an associative response to external stimuli that are involved with systematic cellular behaviors? As stated previously associative learning has been observed many times in other complex multicellular organisms however has yet to be observed in simpler unicellular forms of life. Answering this question was one of the most important in the field of neuroscience.

Related Works:

- **Associative Conditioning Is a Robust Systemic Behavior in Unicellular Organisms: An Interspecies Comparison**
  Carrasco-Pujante J, Bringas C, Malaina I, Fedetz M, Martínez L, Pérez-Yarza G, Dolores Boyano M, Berdieva M, Goodkov A, López JI, Knafo S, De la Fuente IM. Associative Conditioning Is a Robust Systemic Behavior in Unicellular Organisms: An Interspecies Comparison. Front Microbiol. 2021 Jul 19;12:707086. doi: 10.3389/fmicb.2021.707086. PMID: 34349748; PMCID: PMC8327096.

Contributions:

This study has contributed greatly to the study of neuroscience, more specifically to the study of associative memory. This study stands out in this field because of the results it yielded from the experiments that were conducted on unicellular organisms.

Experiment set-up (including metrics, benchmarks, data collection):

Preparations: The experiments conducted in this paper all used the same preparations. The unicellular organisms are placed within an area where they can be stimulated by two separate stimuli , galvanotaxis and chemotaxis, simultaneously. The system is also connected to two electrophoresis blocks, one of these blocks is connected to a standard power source while the other is connected to the first one using agar bridges. The experimental structure was placed on top of the second block, this structure had a sliding glass door that allowed for the placement of cells.

Measurement: Measurement was taken multiple times throughout the experiment to map the trajectory of cells during different parts of the experiment. These measurements were then put onto a graph where they could later be compared with each other to find the results of the experiment.

Procedure: Initially there were 50 amoeba added to the experiment area with no external influences. With no external influence it was determined that the cell migration demonstrated by the amoeba was random and within 30 minutes had explored the entirety of the experiment chamber. Next, there was more amoeba added to the experiment; these were to be influenced by a small electric current of approximately 300 mv. When the current was introduced into the experiment the amoeba had begun to move towards the source of the electric current. Then, 50 more amoebae were tested with their reaction to a chemical gradient. The reaction to this was similar to the electrical current with most of the amoeba healing towards the chemical gradient and completely changing their normal trajectory. The final step was testing what this amoeba would do when subjected to both stimuli at the same time. There were 180 amoebae placed into the experiment area and both stimuli were provided, some ignored the electric current and went to the chemical stimulus while others went straight to the electrical current this had shown that the previous amoeba present through the other steps had now developed some form of associative behavior.

Delivery: Overall four amoeba groups total were used in this experiment the first being the one with no external influence, the second being the one with electrical influence, the third being the one with chemical gradient influence, and the final one being made up of recreated amoeba from the previous two steps.

Results and Discussion:

The results of the experiment concluded that new locomotion patterns had emerged in amoeba when previously none had existed before. Through the use of various groups of amoeba and various methods of testing, it can be confirmed that these amoebae have new patterns of movement where there were none present before.

Conclusion:

In conclusion, the study has proven the hypothesis that amoeba can demonstrate the ability to have associative learning. This study in particular was the first of its kind to successfully demonstrate that unicellular organisms do in fact have associative learning, a thing previously observed to exist in multicellular organisms. This study was a major breakthrough due to the fact that associative learning is possible in more simple organisms and is not solely limited to more complex organisms.

Update after presentation:

After receiving feedback on the presentation I went through all the slides I worked on and reduced the amount of wording in those slides while simultaneously adding more images. Everything that I could not in the slides or during the presentation is in this report and in greater detail.

Pavlov's cockroach: classical conditioning of salivation in an insect.

Motivation:

The main motivation for this study is to determine whether insects (namely cockroaches) are capable of demonstrating conditioning of salivation in response to odors. As there has been extensive research on classical conditioning in humans and dogs. On the other hand, research on insects namely cockroaches points to them having great learning skills and the ability to learn through reinforcement learning (through odor) but proofs of whether cockroaches have the ability to be conditioned are still to be determined.

Problem Statement:

As mentioned above, there is extensive research that indicates that conditioning of salivation is present in humans and dogs as it is an important physiological action that helps to ingest food. On the other hand, there was no basis of proof that the same goes for insects (namely cockroaches). Therefore the following study is targeted toward insects to find evidence of neural control and changes connected to autonomic functions in insects through the conditioning of salivation in cockroaches. Which in turn can provide a base model to study the cellular basis of conditioning in creatures with simple nervous systems (insects).

Related Work:

Watanabe, Hidehiro, and Makoto Mizunami. "Pavlov's Cockroach: Classical Conditioning of Salivation in an Insect." PloS One, vol. 2, no. 6, 2007, pp. e529–e529, https://doi.org/10.1371/journal.pone.0000529.
Key Takeaway: Proved that cockroaches can be conditioned to salivate using classical conditioning

Watanabe, Hidehiro, and Makoto Mizunami. "Classical Conditioning of Activities of Salivary Neurones in the Cockroach." Journal of Experimental Biology, vol. 209, no. Pt 4, 2006, pp. 766–79, https://doi.org/10.1242/jeb.02049.
Key Takeaway: a precursor to the 2007 experiment. This study developed the conditioning time frame and saliva collection for the 2007 experiment. This study looks at how the neurons of an insect (mainly salivary neurons) when conditioned will react and how that correlate to the actual amount that the insect salivated

Watanabe, Hidehiro, et al. "Classical Olfactory Conditioning in the Cockroach Periplaneta Americana." *Zoological Science*, vol. 20, no. 12, 2003, pp. 1447–54, https://doi.org/10.2108/zsj.20.1447.
Key Takeaway: a precursor to the 2006 experiment. This study proves that insects can develop olfactory memories through conditioning

Nishino, Hiroshi, et al. “Projection Neurons Originating from Thermo- and Hygrosensory Glomeruli in the Antennal Lobe of the Cockroach.” Journal of Comparative Neurology (1911), vol. 455, no. 1, 2003, pp. 40–55, https://doi.org/10.1002/cne.10450.

Key Takeaway: uses the odor delivery system developed by Kanzaki R, Ikeda A, Shibuya T in 1994 which is then utilized in the 2007 experiment. It also studies the neuron in an insect when different types of stimulus are presented to the insect

Contributions:

This study has contributed to 40 other publications and studies, and most of the studies use this study as a basis to further examine learning and memory in cockroaches, associative mechanisms, operant conditioning, aversive conditioning, etc.

“Learning and memory in the orange head cockroach (Eublaberus posticous).” *PubMed*, 22 August 2022, https://pubmed.ncbi.nlm.nih.gov/35994454/..

Key Takeaway: The study used the finding from this study to show associative learning and memory in the orange head cockroach

Experiment set-up:

Preparations: Adult male cockroaches (*Periplaneta americana*) with wings removed from a lab colony were used. They were fed a sugar-free yeast extract and drinking water and kept at 26-28 degrees Celsius under a light-dark cycle. The cockroaches were kept at room temperature (1-2 hours) with their cuticles (hard shell) on the neck and labium removed restrained on a wax-coated dish with most of its body parts fixed with wax (legs, neck, dorsal abdomen) and its antennae fixed with staples. The cockroach's salivary duct was cut and inserted into a plastic chamber with the tip covered with Vaseline

Measurement: Measurements were taken more than 10 minutes after the setup and salivation was stabilized. A plastic capillary tube would extract the saliva from the chamber every 1 minute. The length of the fluid column is measured to calculate the volume of salivation

Procedure: 5 forward pairing P+/V- or P-/V+, backward pairing P+/V-, conditioned stimuli alone, and unconditioned stimuli alone trials were conducted. Forward pairing trials consisted of presenting a Peppermint or Vanilla odor to an antenna before presenting a sucrose solution to the mouth. Backward pairing trial consisted of presenting a sucrose solution 3 seconds before presenting a Peppermint odor and subsequently presenting an unpaired Vanilla odor. Conditioned stimuli alone consisted of just presenting the odors. Unconditioned stimuli consisted of just presenting the sucrose solution. The forward pairing, backward pairing, and conditioned stimuli trials are carried out with 4 seconds of exposure to the odor and a 5-minute interval in between the exposure to the other odor while the unconditioned stimuli trials had a 10-minute interval in between the sucrose solutions. Salivation response measurements for forward pairing trials were taken 30 minutes or 1 day after conditioning while backward pairing and conditioned stimuli trials were taken after 30 minutes, and unconditioned stimuli were taken after 40 minutes. P indicates Peppermint odor and V indicates Vanilla odor, +

indicates that the sucrose solution is given, and - indicates when the solution is not given.

Delivery: Apple odor was used as a control group. 2 groups of cockroaches were used for P+/V- trials, their saliva was measured for 30 minutes and 1 day respectively. P-/V+ trials are also following the same procedure.

Results and Discussion:

The results discovered indicated that untrained cockroaches do not salivate in response to the odors except for the apple odor. After pairing the odor (unconditioned stimuli) with a sucrose solution (conditioned stimuli) the cockroaches are conditioned to salivate. They exhibited salivation responses to the sucrose-associated odors. The conditioned response was maintained for a day. Backward-pairing, Conditioned Stimuli alone, and Unconditioned Stimuli alone did not show a conditioned response.

Conclusion

In conclusion, this study verified the hypothesis that classical conditioning is possible in other species such as insects (namely cockroaches). It is also the first study that reliably demonstrated the conditioning of sophisticated neural control of autonomic function in invertebrates which were originally thought to be only applicable to vertebrates only. Cockroaches have relatively simpler brain structures and a relatively small amount of neurons which helps to study neural mechanisms underlying the conditioning of salivation.

After the feedback on the presentations was given, a contribution section was added to the report to discuss the contributions that the paper made. The slides on the other presentation were also modified in order to accommodate for more graphics and less lengthy sentences.