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CSCI 191T Bio-Inspired Machine Learning

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Explanation of Pavlov’s Cockroach and Learning by Association in Plants

The first of the two articles researched was “Pavlov’s Cockroach: Classical Conditioning of Salivation in an Insect”. This article on cockroach salivation took Ivan Pavlov’s idea of classical conditioning and uses the motivation that all phyla, which includes evolutionary related animals, may grow with the same survival abilities. In addition, Watanabe and Mizunami (authors) were motivated to learn if neural control exists elsewhere (in insects such as cockroaches), other than humans and dogs learned by Pavlov. This created the problem statement: Is classical conditioning in salivation fundamental for all organisms applicable, and is it ubiquitous among different phyla? (slide 29)

As mentioned in the presentation (slide 31), simplypsychology.org and frontiersin.org both had informational sources. The first has a concise explanation of Pavlov’s used classical conditioning for dogs, and how positive reinforcement of a neutral stimulus can become a conditioned stimulus. The second source is the same cockroach salivation experiment, but updated 13 years later (2020) by doctors and researchers in Cologne, Germany.

The approach for this experiment was to explore whether these results would provide a useful model system for studying cellular basis of conditioning of salivation in the simpler nervous system of insects.

As for setting up the experiment (also on slides 30 - 31), there were 20-30 adult cockroaches which were fed sugar free yeast extract and as much water needed. They were then maintained in a light-dark cycle (12 :12) and 26-28 degree Celsius habitat. The cockroaches were then restrained ventral side up on low melting wax, antennas made immobile from staples (mouth could move freely), and the Cuticles and labium removed to expose the salivary ducts. Referring to slide 31, saliva was collected on a 200 nanometer slide (vaseline used to prevent spilling), and sucked through a syringe for testing. The cockroaches tested odors such as peppermint, vanilla, sucrose, NaCl, and apple in 40 nanoliter doses through a vacuum, and for gustatory stimulation, 4 nanoliters of sucrose and NaCl solution were presented to the mouth. The odors were presented for 4 seconds at a time (solution introduced on the 3rd second), with 5 minutes between each odor. Results were collected after 30 minutes and 1 day separately. The wilcoxon test was used to compare these sample pairs *W* = [*sgn(x2,i - x1,i)* \* *Ri*], where *W* is the test statistic, is the sample pair size, *sgn* is the sign function, *x* is the ranked pair, and *Ri* is rank. (slides 35, 37-38)

The results were rather promising, as there was steady salivation of 100-200nl, almost 600nl for NaCl and Sucrose, and an average of 300nl for peppermint and vanilla 30 minutes after the experiment, and even less (150-200nl) 1 day after the classical conditioning. Apple odor, sucrose, and NaCl solution were heavily preferred over vanilla or peppermint in control experiments (about 100 nl of saliva more shown in chart on slide 37-38) according to the wilcoxon test, which compares two paired groups. Untrained cockroaches showed no salivation responses to vanilla or peppermint odor, trained cockroaches only had a salivation response change to odor paired with solution, and CS alone and US alone trials in backwards pairing did not induce a conditioning effect.

These results demonstrated that the conditioning of salivation in cockroaches exists in insects just like dogs and humans, supporting the motivation and approach that salivation conditioning is ubiquitous among different phyla.

All contributions to this experiment were designed by Hidehiro Watanabe and Makoto Mizunami, with Watanabe doing the experiment as well as analytics to show conditioning of autonomic function in invertebrates.

The second article researched, “Learning by Association in Plants” by Monica Gagliano, explores the motivation that with high quality resources spread out in the world, survival can be increased by environmental cues, and it is yet to be determined if plants can use this method in a similar fashion as humans do. With that being said, problem statements arise such as: Can associative learning be used by plants/pea seedlings? Are they possibly affected by homeostatic needs like hunger, thirst, and sleep? Do they have a similar learning process to the Animal Kingdom?

There are many related works and with identical concepts of associative learning and foraging listed below the article as references. Doctorate professors like Maxine Whitefield explored floral association in sunbirds, Nigel Raine explored speed and memory in bumblebees, and Jonatan Nilson explored stimulus and food tracking in Atlantic cod (fish).

The approach taken to this experiment is to explore whether plants can also learn through forming associations, since plants can already learn new behaviors through non-association, such as photosynthesis and growth in habituation.

For the experiment, garden pea seedlings (*pisum savitum*) were germinated

hydroponically in 250 mL round containers, kept in the dark in a 5.3 m^2 Controlled Environment Room (CER), soaked in water for 24 hours and then wrapped with clean, wet paper-towel surrounded by an external layer of aluminium foil, and finally placed in a Y-maze with a fan at one end, and blue light at the other. There were 45 pea seedlings, and the experiment lasted 5-8 days long (8-h light:16-h dark cycle). After, they were kept in darkness with the exception of 1-hour light exposures during the three daily training sessions. These sessions had an hour of light, or fan, and 30 minutes of overlapping (light and fan), for a total of 90 mins (training and testing sessions). (slides 42-43)

Results (slides 44-45) after the experiments showed that 62% and 69% of seedlings showed learning (green bar) of following the light. The rest (blue bar) did not learn. However, the control group followed the blue light both times (white bar) (charts on slides 44-45). Referring to graph B on slide 45, circadian effects on behavioral performance of pea seedlings started to change. In graph B*i*, control seedlings grew towards last known light location (control seedlings), while in graph B*ii* and B*iii*, phase-shifts are shown to disrupt phototropic sense.

This concludes that the circadian rhythm in seedlings had deviated from the zeitgeber cycle, with possible phase shifts from light pulses during training days, showing that plants can learn when and where to grow to survive. Shared by both animals and plants, associative learning is a mechanism that is now proven to be universally adopted.

Contributions to this experiment come from grants of the University of Western Australia and the Australian Research Council, as well as other researchers. Researchers C. Cirelli and J. Tomkins provided early discussions, C. Silvano, R. Creasy, W. Piasini and P. Tallai provided technical assistance, B. Radford gave statistical advice, L. Simmons commented on an earlier draft, and S. Whalan provided constructive feedback, as well as 3 other anonymous people.

Presentation Experience

Since my group ended up as the last group to present, with the accumulation of time taken by other groups and the first half of ours as well, two of us group members had to finish our project presentations by recorded video. I also had to learn how to use OBS so I could record my presentation. It felt a bit odd, since everyone else had to present live in class or on zoom, so I recorded my video in one take to be fair. One thing I wish I had put a little more focus on was distinguishing “motivation”, “problem statement”, and “Approach”, as mine in the presentation were probably a bit difficult to follow or distinguish as a viewer and listener. Other than that, I would have loved to describe more statistics and experimental values during the presentation, but there were just too much to easily explain without general simplification, which is what I tried to show in the slides with just charts, graphs, and small notes. I do believe that I should have put more notes by the charts and graphs, because I got a tiny bit lost when trying to explain everything verbally. Being the last presentation and having to record a video alone was a bit hectic overall, but we ended up getting everything complete and are happy to be done with it.

Works Cited

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