Associative Conditioning in Moon Jellyfish: Behavioral Adaptations During Feeding

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ABSTRACT & INTRODUCTION

Pavlov's Dog Experiment demonstrated that dogs couldlearn through a "feeding training period" and associate sound with food illustrated through memory recall, but can moon jellyfish display the same behavior? Some studies indicate that jellyfish can learn – known as associative conditioning – despite not having a brain. Moon jellyfish, also known as Aurelia aurita, have a complex neural system with a large and small network, possibly giving them the ability to "learn" and to respond to a certain stimulus over time. There have been studies on conditioning and behavioral capabilities of other organisms such as planaria (Deochand, 2018), but studies on invertebrates, specifically jellyfish, are generally limited. While box jellyfish have been found to have an aptitude to be conditioned (Bielecki et al., 2023), other jellyfish species such as moon jellyfish are not as widely researched for conditioning. This project's goal is to bridge that gap, investigating whether moon jellyfish can 'learn' through conditioning and respond to environmental stimuli in a way that parallels more cognitively complex animals. We found that jellyfish pulse rates under all tested conditions decreased over the 9-day trial, but the pulse rate of jellyfish under red light before feeding grew closer to the pulse rate of jellyfish during feeding each day, showing that conditioning may have occurred.

ABSTRACT & INTRODUCTION

HYPOTHESIS

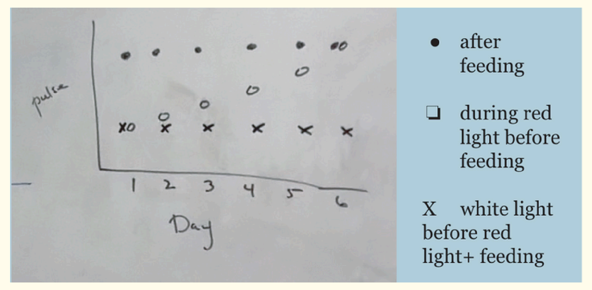

Figure 3. We hypothesized that over time, the pulse rate of jellyfish ephyra under red light before feeding would increase, growing closer to the pulse rate of jellyfish during/after feeding. This is based on the assumption that jellyfish demonstrate increased pulse rate during feeding. We predicted that the pulse rate of jellyfish under white light before feeding and jellyfish during/after feeding would remain constant across the 2-week experiment.

METHODOLOGY

The **independent variable** is whether the jellyfish were exposed to red light at the time of feeding.
The **dependent variable** is the pulse rate of the jellyfish over 2 weeks of training when exposed to red light but not brine shrimp.
All experiments took place on a light board with regular (white) or red lamps shining from above. Jellyfish ephyra were stored in 6 well plates, with one jellyfish in each well. Outside of data collection, the plates were stored on a shaker rotating at a constant rate.
Control Group: brine shrimp alone, red light alone (3 days) (n=3). For both procedures, we timed for 5 minutes and record, then counted the ephyra pulses per minute and logged the data in the spreadsheet.
Experimental Group: red light with feeding (every weekday for 2 weeks) (n=3). Check the salinity of the solution, ensuring that it is in the 32-36ppt range, optimal for ephyra. We shone a regular light lamp above the plate for one minute, checking a baseline pulse rate. The 6-well plate is placed on top of a light board shinign regular light.
TESTING period: We turned on the red light for 5 minutes and observed the pulse rate of jellyfish in the 6-well plate with red light in the absence of brine shrimp. This demonstrates the "conditioned" reaction of the jellyfish to red light.
TRAINING period: We used a micropipette to add brine shrimp and collected data for 5 minutes (observe the pulse rate of jellyfish who were fed brine shrimp in red light). We then took away the light and returned the plate to the shaker.

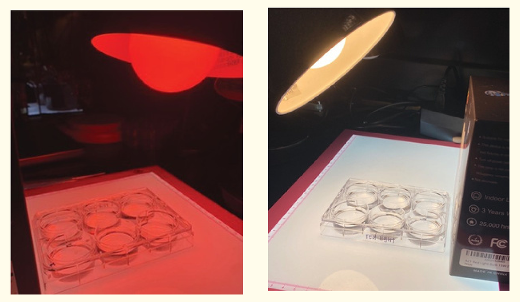

Figure 1. Our experimental setup consisted of 6-well plates, a light board, and lamps with different colored bulbs (white/yellow and red). Plates were stored on a shaker when we were not collecting data/training.

Figure 2. Our code used OpenCV to draw contours around the jellyfish and detected changes in area to count pulses.

Videos were recorded using cell phones and color filtered using iMovie, because the red light washed out the jellyfish, making them hard to see. We developed a **Python program** using the **OpenCV** library to streamline the pulse rate counting process, but we found that this method was only accurately for jellyfish under regular light because the red light washed out the shape of the jellyfish. The program takes a 1-minute video of the jellyfish and draws a contour around the "blob," finding peaks in area to estimate pulses. We went through an extensive trial and error process, using filters, thresholds, and other methods to improve accuracy.

RESULTS

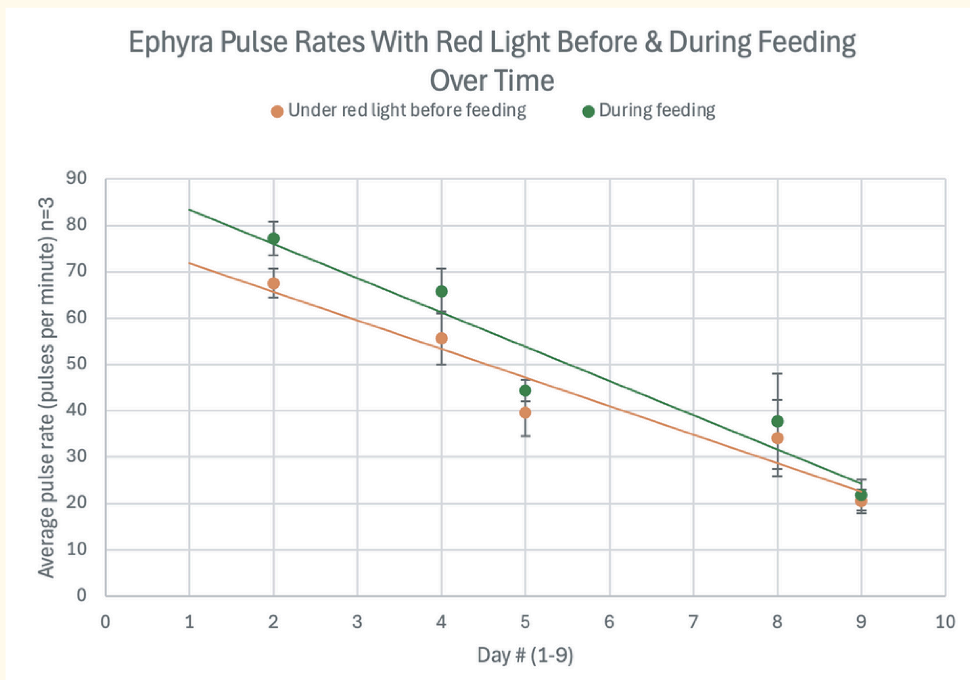

Figure 4. Ephyra pulse rates generally decreased over the 9 days of the experiment. However, the pulse rate of jellyfish under red light before brine shrimp feeding and the pulse rate of jellyfish during feeding (after brine shrimp were pipetted into the wells) on any given day grew closer as time progressed. This is demonstrated by the green and orange points growing more similar on later days (8 and 9) than on earlier days (4).

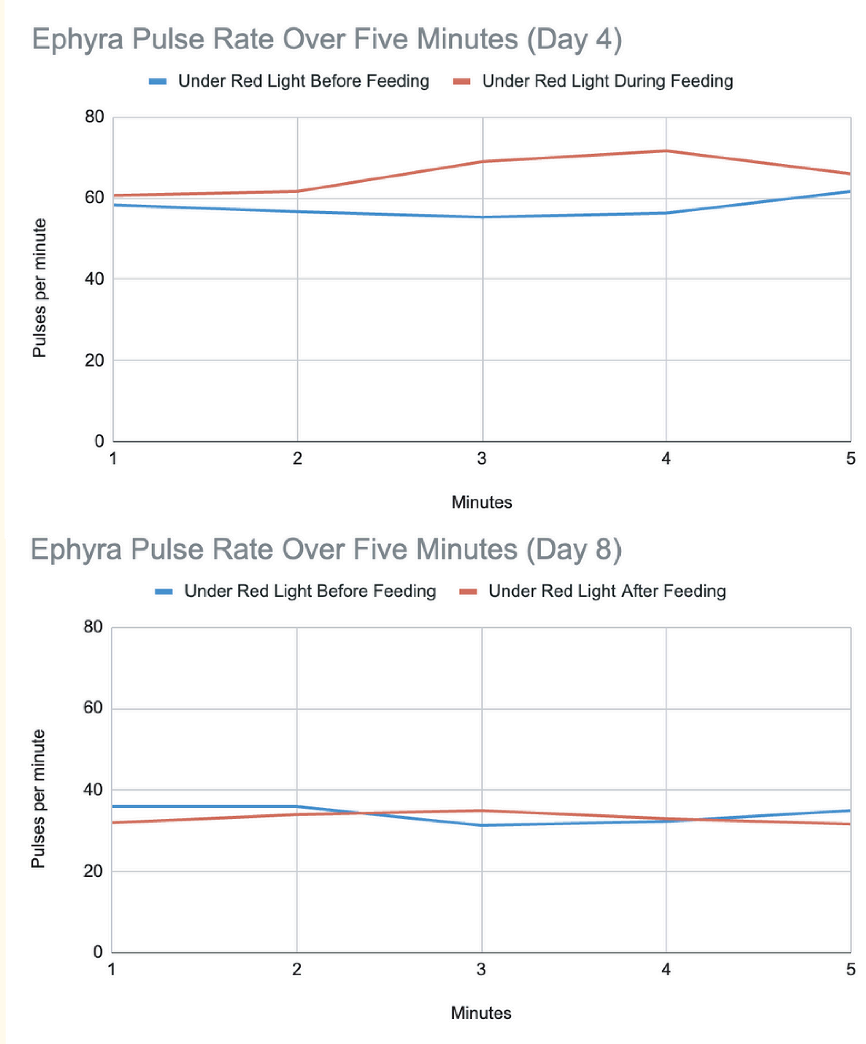

Figure 5. Ephyra pulse rate over 5 minutes under red light before feeding differed from pulse rate under red light during feeding earlier on in the trial (day 4, top). Later in the trial on day 8 (bottom), the pulse rates of jellyfish before and during feeding were more similar.

While our results did not demonstrate that the ephyra showed pulse rate increase in response to the red light, their pulses generally decreased over time, and the pulses with red light before and during feeding became more and more alike over the 9-day trial. Near the beginning of the trial, there was a statistically significant difference between the pulse rates of jellyfish under red light before and during feeding, but at the end, averages grew closer and error bars overlapped, meaning the jellyfish behaved more similarly before and during feeding. Therefore, jellyfish may have experienced conditioning, displaying feeding behaviors under red light, even without being fed.

DISCUSSION

Our results demonstrate that moon jellyfish may display associative conditioning. The pulse rate of ephyra generally decreased over 9 days (in all conditions - regular light, red light no feeding, red light during feeding). This may have been due to ephyra aging or the fact that they were held in isolation in 6-well plates, different from their original environment in cones.
Although pulse rate decreased overall, the pulse rate of jellyfish with red light before feeding occured was significantly different from the pulse rate during feeding at the beginning of the trial. Over time, these values grew closer, going from a 9.63 average pulse difference (day 2) to a 1.3 pulse difference (day 9). This means jellyfish began to display similar behaviors to feeding when exposed to red light over time. Therefore, the associative conditioning (or jellyfish behaving as if they were being fed in the presence of red light, even if they were not red yet) portion of our hypothesis was supported, despite pulse rates for all conditions generally decreasing over time. If later studies also find that Aurelia aurita can undergo associative conditioning, this challenges our current understanding of decentralized neural networks in invertebrates, and jellyfish nerve nets should be explored more thoroughly. A better understanding of jellyfish behavior paves the way for ecological decisions and projects. For example, we could apply conditioning techniques to keep jellyfish out of overfished areas or develop learned avoidance from human nets, boats, etc. Research on jellyfish behavior also provides a better understanding of how jellyfish respond to environmental stressors, such as climate change.

FUTURE RESEARCH DIRECTIONS

Possible sources of error include jellyfish being held in isolation for this experiment (different from their normal environment), slightly inconsistent numbers of brine shrimp, human error during data collection and counting, and different initial pulse rates of ephyra. Future experiments could aim to limit these conditions. Other species of jellyfish could also be researched for signs of conditioning. Stimuli other than red light (i.e. scent, turbulence, touch, sound) and behaviors other than feeding (movement, symmetry, obstacle navigation) could be explored in moon jellyfish. Our Python OpenCV model to detect pulse rate can be further improved using machine learning to adjust to different lighting conditions of videos and to improve accuracy.

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