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DISCRIMINATION IN PLANTS: LEARNING THROUGH CONTEXT-SPECIFIC BEHAVIORAL STIMULATION OF THE MIMOSA PUDICA

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Synopsis:

Experimental stimulation of the Mimosa pudica leading to discrimination between non-harmful stimuli as a form of adaptive context specific learning. This is shown through the ability to distinguish and progressively learn to not engage in species typical behaviors towards a singular stimulus. Also show as not being simple mechanical exhaustion through the reemergence of the same species typical behavior when presented with another non-harmful stimuli.

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Abstract

The utility of the *Mimosa pudica's* visible reaction, when presented with a stimulus, serves as a method for testing its specific learning capabilities. The behavior of the *Mimosa pudica* leaf folding as a defensive mechanism and eventually reduction of that behavior after repeated disturbances from the same stimulus show some form of necessary adaptive learning. The *Mimosa pudica* can habituate to a particular stimulus such that, over time it displays learned non-responsiveness. Even after it has determined to reduce or null its response to a specific stimulus, it can still react, and thus discriminate, fully to a different stimulus. This also allows experimenters to show that the non-responsiveness is not due to a simple mechanism of exhaustion on a biological level. Also, it may allow for researchers to hypothesize that this is due to the plant's natural instinct to increase its survival benefits by reducing the costly action when optimal. Using varying models of previous experiments and our experimental design, we show that over time, the *Mimosa pudica* displays a form of learning through the combination of associative and behavioral learning as evidenced by habituation to varying stimuli which are not due to simple mechanical exhaustion.

Keywords: *Mimosa pudica*; Habituation; Learning; Discrimination; Generalization

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The study of memory systems and the mechanisms of learning is a major part of the study of psychology within living organisms. Understanding how the underlying factors of learning work, helps one understand the complexity of an organism's behavior as a whole. By understanding how such mechanisms function in more simple organisms (simple at least regarding psychology), one can try to understand more about how memory and learning serve a function in the life of the organism. While learning is a broad term that can be tailored and conceptualized differently among various life forms, the basic tenets and core mechanisms are mostly universal. This basic form of learning that has been shown throughout the spectrum of life forms, from complex to simple, has been classified as associative learning which leads to adaptive change in an organism's behavior. It is theorized to exist in almost all cell types, even those without a nervous system such as single-celled eukaryotes and plants (Sorek, Balaban, & Lowenstein, 2013). What we are looking at from this experiment, is whether or not the botanical subject of our experiments are capable of a form of learning more complex than mere association, as would be shown by discrimination.

The *Mimosa pudica*, also known as the "touch me not" and shame plant, is a useful plant for study, because of its easily observable response to external stimuli. This unique property of the *Mimosa pudica* is its response to external stimuli, such as physical touch, by folding the leaflets of the stimulated leaf and drooping of its stem. While reactive responses are seen in other botanical species, this particular function of the *Mimosa pudica* has been inferred to serve multiple purposes of protection and the optimization of photosynthesis.

This paper looks at this specific function from a psychological lens. Our experimental study was to determine if there is a significant difference in rate of habituation when varying stimuli stimulate the plants and if there is any difference in rate of closure when presented with a new stimulus.

Environmental Factors

Survival is the base function of biological organisms. To most efficiently do this, plants need to develop optimal defense mechanisms. The *Mimosa pudica's* defense mechanism of rapid leaf folding, triggered by abiotic stimuli-environmental factors that can act as a stressor (Thellier & Lüttge, 2013) has various beneficial factors to the plant survival. The reasons as to why the *Mimosa pudica* does this has been explained by various researchers as reducing the size of the leaf and its overall visibility (Braam, 2005), the revealing of the *Mimosa pudica'*s defensive spines, which may deter predators (Eisner, 1981), and finally the leaflets have been theorized to close so that insects will not be able to land on them (Pickard, 1973). However, these plants have to pay a cost to repeatedly close and reopen their leaves because of the energy it requires to trigger its leaf closure. By closing their leaflets, the plant is reducing its photosynthesis rate by about 40% (Hoddinott, 1977). This constant closing may lead to decreased amounts of energy in the plant, which may cause the plant to suffer. What determines when the leaflets should close and reopen? The *Mimosa pudica* needs to be able to discriminate or respond differently to varying stimuli. In this case, it would be to distinguish between damaging and non-damaging stimuli (Amador-Vargus et al., 2014). The best way for this to happen is for the plant to reduce its level of responsiveness to repeated, stimuli similar to processes of habituation (Groves & Thompson 1970). By becoming habituated to a particular stimulus, the plant is better able to distinguish when it should close or open to the correct level, so as to optimize their standard of photosynthesis production. Amador-Vargus et al., (2014) showed in their paper, regarding leaf closure responses, that there is this tradeoff between defense and photosynthesis. This was shown through measuring the leaf reopening and the intensity of the closing of the leaf, and observing that repeated non-damaging stimuli produced quicker opening times, and less intense, or no reactions via habituated responses. Unlike in the previous study, we did not distinguish as to whether or not the presenting stimulus was damaging or non-damaging, but more loosely classified them into either natural or unnatural. A natural stimulus would be those types of stimuli that are found in a naturalistic or real world setting. An unnatural stimulus is one that would never be found in nature, and would only be found in an artificial environment (i.e. dropping the entire plant or adding an electric shock). We still aimed to test whether or not there would be a difference in closure rates between these two classification systems, similarly to what the Amador-Vargus et al.'s (2014) experiment examined.

Biological Factors

The biological mechanism for this change are studied extensively, and there is varying ideas as to how exactly it happens, and why. The movements of the *Mimosa pudica* in response to stimuli seem to be caused by electrical, hydrodynamic, and chemical signaling (Volkov et al., 2014). The overall accepted hypothesis for why this is happening, is because of the changes in the turgidity, or water pressure, in the plant cells of the pulvinus which is the base of the leaflets (Kapadia, 2004). The contraction of leaflets are caused by the loss of water pressure in the cells of the pulvinus causing a loss of internal pressure and the leaflets to fold up and droop down (Allen, 1969). This causes a redistribution of the water from the upper and lower parts of the pulvinus, which given enough time reverses and causes the leaflets to reopen. This happens when the K^+ ions are removed from the leaflet, and there is an influx of $Ca2^+$ ions which draws out the water and the leaflets lose their rigidity. It should be noted though that the change does not pass into the pulvinus and thus it does not trigger other leaves on the plant to fold up. This does not hold true in our unnatural condition in which we shocked the entirety of the plant, rather than in the natural conditions in which we only stimulated certain leaves. For the leaflet to reopen, there needs to be an influx and redistribution of K^+, and is hypothesized that a change in K^+ levels within the cells affect the osmotic potential and thus affect the turgor pressure (Campbell & Thomson, 1977). In addition to this, previous studies have tested and hypothesized that this calcium bases signaling system can be representative of simple models of plant memory (Bose & Karmakar, 2008).

Psychological factors

A conventional definition of learning such as; “learning is an enduring change in the mechanisms of behavior involving specific stimuli and/or responses that result from prior experience with similar stimuli and responses” (Domjan1998), is adequate for understanding learning on a basic level. However, a more complex definition is required to be able to see how other organisms might exhibit signs of learning. Lachman (1997) suggested that a better definition of learning might be; “the process by which a relatively stable modification in stimulus-response relations are developed as a consequence of functional environmental interaction via the senses.” Learning happens through a myriad of ways. One underlying mechanism of learning is habituation, which is, “decreased response to repeated stimulation” (Groves & Thompson, 1970). Gagliano et al.’s (2014) recent work on determining rates of learning and forgetting depending on various environments provided some of the strongest and most recent evidence of sustained and lasting habituation in the *Mimosa pudica*. They argued that the habituation displayed in the *Mimosa pudica* represents a simple form of memory as shown through sustained behavioral learning of non-responsiveness. This mechanism is a major piece in other studies that have looked at the learning abilities of plants (Amador-Vargas, 2014; Holmes & Gruenberg, 1965; Holmes &Yost, 1966; Simón, 1978). Another key piece of learning is generalization, the tendency to respond to other stimuli that are similar to the training stimulus. Generalization was demonstrated initially through the work of John Watson and his research assistant Rosalie Raynor and their work with Little Albert (1920). Discrimination is a major component of habituation and is exhibited in an organism if "… it responds differently to two or more stimuli" (Domjan, 1998). This idea of discrimination, or what used to be called differentiation, goes back to Ivan Pavlov and is explored in his paper called *Conditioned Reflexes: An Investigation of the Physiological Activity of the Cerebral Cortex* (1927). With all aspects of learning, there is another component to keep in mind, and that is biological preparedness. Martin Seligman defines biological preparedness as, "an innate (inborn) tendency to learn certain kinds of associations relatively" (1970, p. 71). Seligman's work can be applied to the current research. Even though, Seligman's work focused mostly on the development of human phobias, because the current study seeks to examine the *Mimosa's* "innate (inborn) tendency to learn certain kinds of associations." When we talk specifically about plants and their biological preparedness, we are talking about natural innate abilities that they have received from previous generations that would allow them to survive best, thrive and reproduce during their time. This is similar to what Gagliano et al. (2014) were describing when they talked about, "a fixed 'blueprint," and how the induced adaptive responses is ingrained in the "behavioral repertoire of an organism."

This current study seeks to confirm habituation of the leaf folding response in the *Mimosa pudica* to specific stimuli and to determine if discrimination by the plant is possible. In addition to this, we hypothesize that this effect are seen in all three of our conditions of touch, water, and drop and that their rate of adapting to their stimulus will not differ. Finally, we hypothesized that this reduction of the biological response is not due to simple mechanical exhaustion in all conditions and that exposure to a new stimulus will produce closure rates similar to the first day of testing regardless of the type of stimuli. If the plant's reactions to new stimuli are similar to their original condition's first day of testing, then this can be seen as a sign of discrimination because the plant's learned reaction would not be transferring over to a different stimulus as would be seen with a generalization effect.

Materials and Methods

Design and set-up

The entirety of the experiment was done in an 8 ft. x 10 ft. windowless room. The light used to stimulate sunshine were two; Sun Blaze® 4 ft., T5 HO 6500˚k "blue" lamps with aluminum reflectors so as to maximize output and diffusion. They were suspended above the plants at the height of three feet. The seeds were ordered from Seed Savers Exchange. Each seed was soaked in water for 24 hours as per directed on the packaging. The seeds were then transferred to one of three 8 inch x 12-inch individual planters in which they were separated and individually placed in Grodan Macro plugs for sowing. They were then placed inside of the experiment room on top of an elevated grow stand. This stand raised the plants approximately seven inches off the ground. The lights were kept on for a 24-hour cycle throughout the experiment. This meant that there were no periods of dark in the growth room due to our intent of helping optimize growth to maturity. Due to trying to maintain consistency in our overall experiment, we did not change this cycle throughout our experiment. This was also done so as to have as much experimental control as possible. If we had introduced a dark cycle, the plants would have reacted to it by closing every dark period, and thus adding a variable that we would not be able to control. Temperature and humidity were measured consistently throughout the experiment. The average temperature remained consistent at 28.13 degrees Celsius with a high of 31.67 degrees Celsius and the low of 22.22 degrees Celsius. The humidity averaged approximately 39% throughout the experiment with a high of 58% and a minimum of 29% humidity. The plants were watered every three days with 700 mL of water in each plug.

Sowing

18 days after the seeds were sown; we transferred 15 plants to one of three separate containers maintaining equal spacing. These plants were chosen due to their size and apparent healthiness and maturity. These plants did not vary significantly in size, and the range was 20-30 cm tall. Then transferred into larger pots with the addition of Nature's Care potting mix. The rest of the plugs were maintained in case any extraneous testing was to be conducted with them. The plants were watered solely at ground level so as to not "trip" the plants before the start of testing. Four weeks later, one of the extra plants was tested to see if it reacted, thus informing us that we could begin testing on all other plants. The plant showed no reaction and another extraneous plant was tested two weeks later. It reacted, and at that point testing was able to begin.

Testing set-up

It took a total of 60 days for the plants to grow from seeds to reactive maturity. The experiment consisted of three conditions. All three of containers/conditions had five plants. Only 15 plants were used because of limited overall space for growth and testing, due to the availability of testable plants at the start of the experiment, and to funding limitations. All conditions are based on previous research studies conducted on stimulating the *Mimosa pudica's* leaflets. The three conditions were: touch (cond. 1), water (cond. 2), and drop (cond. 3). In the first condition, the leaves were stimulated using a camel hair brush similar to experiments done by Holmes and Yost (1966) and classified as natural. In condition 2, a pipette was used to administer four drops of water on the leaves tested, similar to experiments done by Holmes and Gruenberg (1965) and classified as natural. For condition 3, the entirety of condition 3's container was raised so that it was three inches off of the growth stand, and then dropped so as to elicit the folding of the plant's leaves, similar to the stimulation done in the experiment by Gagliano et al. (2014). Condition 3 is classified as an unnatural condition because of how unlikely this stimulation would occur in nature. During testing of condition 3, condition 1 and condition 2 were removed from the stand so that they would not be affected by condition 3's impact. This removal only took place after testing per trial had been done on condition 1 and condition 2, when the leaves we were testing were still closed or non-reacting. Observation of particular leaves not tested did not show any indication of closure due to removal, nor did any non-reacting leaves close posttest during removal. This held true when replacing the plants back on the stand.

Procedure

The experiment consisted of five single days of testing beginning at 10 am Pacific Standard Time. Each day consisted of 12 individual tests of each plant in each condition. A series of 12 trials were chosen because it represented a baseline for all three conditions due to all 15 plants ceasing to react at that time. When looking back on previous studies, we could not find a uniformed number of trials that seemed to be consistent, so we used 12 because that was the best fit for our plants. After each test, we measured the length of time that it took for the leaves to reopen after stimulation, before starting the following test. Recording of data is based on the level of the closure of the plant leaves. We identified four levels of closure which were based on similar measurements recorded by Amador-Vargus et al. (2014). Complete closure was noted when all the leaflets closed entirely. The partial complete closure is identified as the leaves moving as one but with a space visible between the leaflets. Partially closed, was noted if the leaflets did not move together or with very little closure. None was recorded if there was no reaction. The average time for the leaves to reopen after a trial was 30 minutes. Each day of testing is followed by a week of non-testing and just maintaining plant care. Testing lasted a total of five weeks with five days of complete testing. A plant from condition 1 was removed after the second day of testing due to noticeable withering and decreased health in the plant. There were no other changes in testing procedures.

At the culmination of the experiment, the plants were tested to see whether or not they had succumbed to simple fatigue or testing effects. All of the conditions were alternated so that the plants were all receiving a new stimulus. The plants were then administered the appropriate stimulus, and the results were measured. The new conditions and their classifications were: Touch (natural) alternated to Drop (unnatural), Drop alternated to Water (natural), and Water switched to Touch. In addition to this, the rate of the leaflet to reopen was timed. The average length of time it took for the plants to reopen was approximately 25 minutes, five minutes faster than at the start of our experiment. The follow-up testing to determine the role of mechanical exhaustion showed results identical to Day 1's testing results.

Results

Sample Characteristics

Each of our conditions started off with five plants all receiving one of the three stimuli. It is hypothesized that there would be significant differences in leaf closures from Day 1 to Day 5, such that Day 5 closure would have a significantly decreased rate of closure than that of Day 1 for all 3 conditions. This would be shown in the *Mimosa pudica* by a significant reduction in its species-typical response of leaf closure. In addition to this, we hypothesized that there would be no differences between conditions with regards to closure rates. Finally, we hypothesized that there would be no difference in closure rates in the follow-up testing and alternating stimuli than compared to Day 1. The data was analyzed using a mixed-design ANOVA with a within-subjects factor of measurement day and a between-subjects factor of condition (touch, water, and drop) as shown in Table 1. Mauchly's test of sphericity was significant, thus indicating that the assumption of sphericity is violated ($X^2(9) = 36.54$, $p < .001$), therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = .49$).

A significant within-subjects main effect is found for measurement day, $F(1.96, 21.64) = 67.54$, $p < .001$, $\eta_p = .86$, such that closure time decreased across measurement days as shown in *Figure 1*. The between-subjects main effect of condition, however, was not significant, $F(2,11) = .89$, n.s., thus indicating no significant difference between the three experimental conditions.

Discussion

The results of the current study, and previous studies show that the *Mimosa pudica* clearly has the abilities of habituation. As shown by the results of the ANOVA, as the trial days progressed, the number of trials it took for a non-response decreased. This is important because it shows that the *Mimosa pudica* had habituated to the stimulus that is was presented with. This rate of habituation was similar in all three conditions regardless of the classification of the stimuli or the stimuli itself. This evidence supports our hypothesis that when presented with continued stimulation, the rates of habituation will not significantly differ. Evidence for habituation to a specific stimulus is shown by the fact that in the follow up phase of testing, where the new stimulus was presented, the plants showed an identical responsiveness to the first trial day in that there was the complete closure of leaves to the stimuli. The total closure to the new stimuli supports our hypothesis and the fact that mechanical exhaustion of the biological processes in the plants was not a factor. If it were due to mechanical fatigue, similar responses as to those on trial Day 5 would be seen. If there had been anything less than complete closure we might have said that there was a generalization effect. Although, the change in the reopening speed for the leaves, might still indicate that there was some generalizability in the plant's reduction of reopening times so as to increase photosynthesis benefits, we cannot conclusively say for sure. However, the fact that there was not a carryover in lack of closure from similar stimuli classifications and in differing classifications show a more discriminatory response to the new stimuli. This complete reaction to new stimuli might indicate some level of discrimination on the part of the *Mimosa pudica*, but more testing would need to be done to confirm.

The gradual non-responsiveness to a single stimuli from trial to trial indicates that there was gradual habituation. There was some sort of learning to the singular stimuli when we are using the aforementioned definition of learning, "the process by which a relatively stable modification in stimulus-response relations is developed as a consequence of functional environmental interaction via the senses" (Lachman, 1997). We can make an argument that the complete reaction to the new stimuli after complete non-responsiveness to the old does show an ability to differentiate between stimuli, and thus show a level of discrimination. However, to more efficiently and assuredly say that more testing will need to be done to better understand the full capabilities of this process in this specialized plant.

Habituation to, and discrimination of different stimuli, are abilities shared by many organisms. The current study can show and support the idea that there are common psychological traits, even in psychologically simple organisms like plants. Further research can elucidate what the biological systems are required to utilize these abilities. What can be inferred from this study is that the biological systems necessary to exhibit these mental skills may be more basic than previously thought.

There were some limitations of the current research. Firstly, the setting of the study was not naturalistic; the plants were exposed to a light source for 24 hours a day. This was due in part, as mentioned previously, to help the plants grow to maturity, and due to lack of funding for either timed or stimulated day cycle lights. We started testing during the 24-hour light cycle and thus felt that it would compromise further trials to switch during our testing period. Another limitation was that the sample of plants were quite small, and the plants were grown entirely separate; another condition that might not exist in a natural setting. One of the bigger limitations was the lack of ability to continue with the follow up testing. This was due in part to the age of the plants by the time this testing was possible and that the plants were showing signs of wilting and some of the leaflets were beginning to fall off. Continued testing would not have been as fruitful as the previous testing and thus testing was discontinued.

Conclusion

The results of the current study, and previous ones, support the stated hypothesis that the *Mimosa pudica* will exhibit habituation to certain stimuli, and maintain said non-responsiveness, as shown in previous experiments (Applewhite 1972; Gagliano et al. 2014; Holmes & Gruenberg 1965; Holmes & Yost 1966). Although this does not demonstrate that the *Mimosa pudica* has similar learning abilities of other organisms, it does show that certain facets of learning are shared between the *Mimosa pudica* and more complex organisms. The current research is a good continuation and expansion on previous research into the psychological abilities of plants. This study coupled with the past and future research will help to determine unique and shared psychological properties of animals, plants, and various other life forms.

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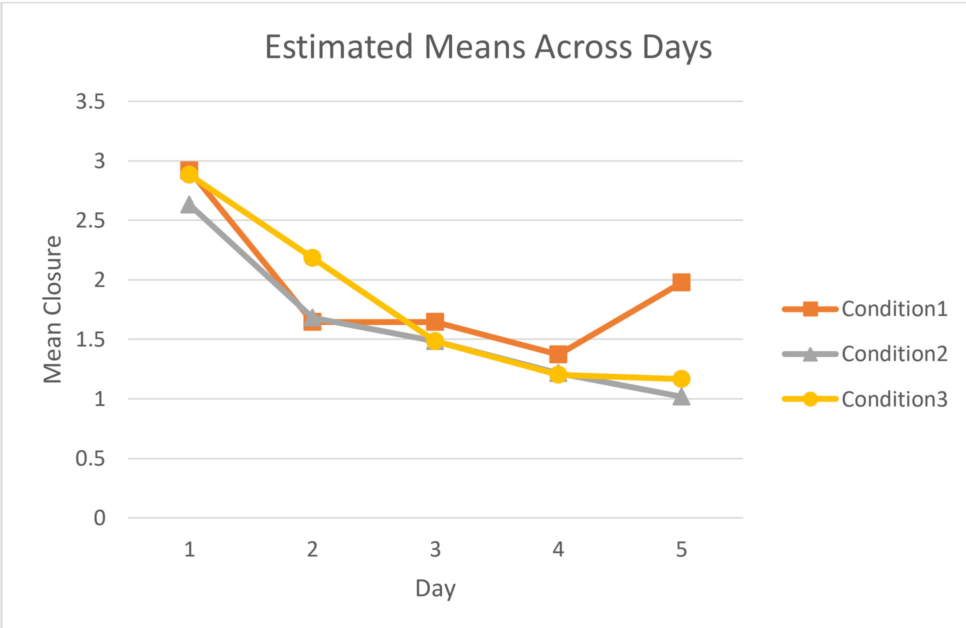
Table 1

Test of Within-Subjects Contrasts					
	Type III Sum of Squares	df	Mean Square	F	Sig.
Day					
Linear	16.203	1	16.203	113.066	0.000
Quadratic	4.878	1	4.878	60.65	0.000
Cubic	0.106	1	0.106	1.279	0.282
Order 4	0.205	1	0.205	19.906	0.001
*Day*Condition*					
Linear	1.173	2	0.587	4.094	0.047
Quadratic	0.745	2	0.372	4.631	0.035
Cubic	0.227	2	0.113	1.373	0.294
Order 4	0.34	2	0.17	16.503	0.000
Error (Day)					
Linear	1.576	11	0.143		
Quadratic	0.885	11	0.080		
Cubic	0.909	11	0.083		
Order 4	0.113	11	0.010		

Figure Caption

Figure 1. The mean level of response in the Touch Condition, Water Condition, and Drop Condition over the testing days.

Figure 1.


Estimated Means Across Days
Mean Closure
3.5
3
2.5
2
1.5
1
0.5
0
1
2
3
4
5
Day
Condition1
Condition2
Condition3