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VIEWS AND COMMENTS

Insufficient evidence for habituation in *Mimosa pudica*. Response to Gagliano et al. (2014)

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Abstract

Gagliano et al. (Oecologia 175(1):63–72, 2014) reported that *Mimosa pudica* habituates to repeated stimulation, as shown by a reduction in response, dishabituation, and stimulus specificity. I argue that Gagliano et al.'s data show an absence of dishabituation, that their experimental design needs an additional condition to test whether there is stimulus specificity, and that most of their data can be explained by motor fatigue. Some data are not easily explained by fatigue, and I suggest a further analysis that may clarify the issue. The status of habituation in *Mimosa* remains uncertain.

Keywords Minimal intelligence · Habituation · Non-associative learning · Plant behaviour

The study of minimal intelligence (Calvo and Baluska 2015; Garzon and Keizer 2011; Reid et al. 2015) is concerned with what computations are possible with little or no brains. Slime moulds can forage from two food sources simultaneously so as to balance protein and carbohydrate intake (Dussutour et al. 2010), pea roots anticipate future nutrient levels (Shemesh et al. 2010), and even the temporal integration of concentrations in bacterial chemotaxis can be seen as a rudimentary form of memory (Segall et al. 1986). It is then a reasonable question to ask whether learning, too, occurs in non-neural organisms. Habituation is a simple form of learning.

Gagliano et al. (2014) argued that the defensive leaf folding behavior of *Mimosa pudica* shows habituation to repeated touch. For the definition of habituation, they cite Rankin et al. (2009) and Kenzer et al. (2013). Rankin et al. defined habituation as "a behavioral response decrement that results from repeated stimulation and that does not involve sensory adaptation/sensory fatigue or motor fatigue." Gagliano et al.'s data clearly show that response decrement, Rankin et al.'s characteristic 1. Plants kept in high-light conditions also show characteristic 2 of habituation, spontaneous recovery of the response over time, here 6 days. To demonstrate that this decrement is learned, it is necessary to distinguish habituation from a mere lack of the physiological resources to detect or respond to stimulation, i.e., sensory or motor fatigue. Gagliano et al. focused on characteristics 7 and 8, those which Rankin et al. identified as most relevant to distinguishing habituation from fatigue: "Traditionally, habituation has been distinguished from sensory adaptation and motor fatigue by the process of dishabituation; however, this distinction can also be made by demonstrating stimulus specificity (the response still occurs to other stimuli)". Gagliano et al. omitted testing characteristics not relevant to distinguishing habituation from fatigue, namely, characteristics 3–6, which are concerned with rate of habituation as a function of various parameters of the stimulation regime, and also characteristics 9 and 10, concerned with long term and higher order effects. They did include a test not mentioned by Rankin et al. a manipulation of energy reserves through light levels.

Gagliano et al. did not strictly follow Rankin et al.'s terminology. Rankin et al. (2009) defined dishabituation in Sect. 3.8.: "Presentation of a different stimulus results in an increase of the decremented response to the original stimulus. […] It is important to note that the proper test for dishabituation is an increase in response to the original stimulus and not an increase in response to the dishabituating stimulus". An increase in response is reduced leaf openness, i.e., increased leaf folding. The dishabituating stimulus is the shaking, marked "DIS-hab" in Gagliano et al.'s Fig. 2. The

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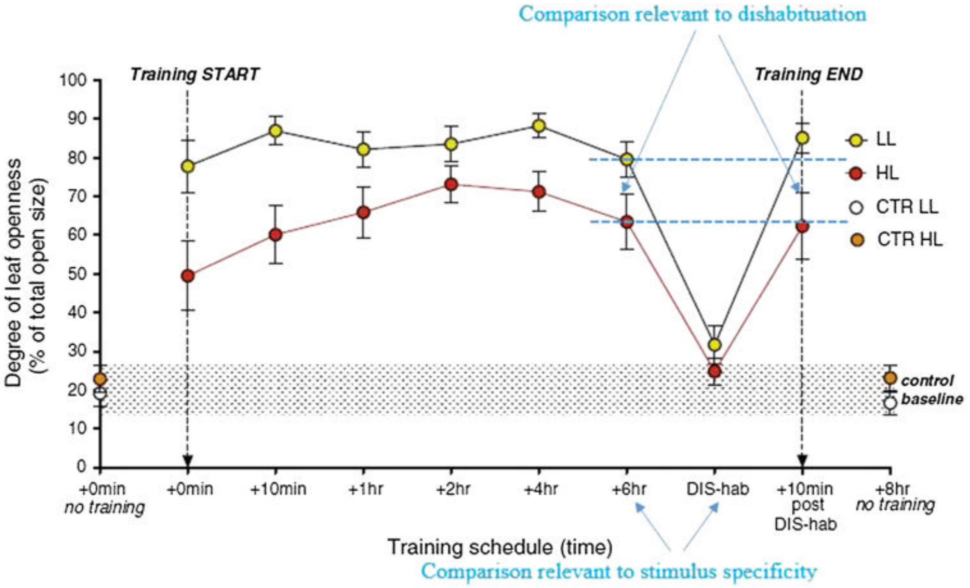

Fig. 2 Habituation training profiles in high- (*HL*) and low-light (*LL*) environments. In a single training day, each plant received the first train of 60 drops at time zero (+0 min) and the second train 10 min later, a sufficient inter-train interval to allow all plants to spontaneously recover and fully re-open their leaves. Subsequent trains were then delivered 1, 2, 4 and 6 h later. Immediately after the last train of drops was delivered (at +6 h), individual plants were shaken at 250 r.p.m. for 5 s (*DIS-hab*). Following spontaneous recovery, plants were administered a final train of drops (+10 min *post-DIS-hab*). Throughout this experiment, a total of 20 naïve plants (*CTR*; $n = 10$ per light environment) were also tested by delivering one drop only in the morning of training (i.e. control baseline indicated by the *dotted grey area*). There were differences between inter-stimulus intervals (ISI) levels for the LL treatment ($P < 0.0001$, $\chi^2 = 21$), but not for the HL treatment ($P = 0.5$, $\chi^2 = 1.2$); for clarity this plot shows data for both ISI levels pooled. The maximum leaf breadth at the end of a train of drops is expressed as a percentage of the maximum breadth prior to the delivery of a train (i.e. degree of leaf openness) and shown as mean ± 95 % confidence interval (CI)

Fig 1 Blue marks text and graphics added to the original Fig. 2 of Gagliano et al. (2014). Arrows and text mark which comparison are relevant to which claimed finding. Stippled lines show the response levels in low-light (LL) and high-light (HL) groups that are relevant to dishabituation. The low-light group folds its leaves less after the dishabituating stimulus; it does not restore leaf folding. The high-light group shows numerically lesser openness, meaning restored leaf folding, but averaged across groups, the change was not significant (color figure online)

habituating stimulus is dropping the pot and plant. The last drop before the shaking is marked "+6 h", and the first drop after the shaking is marked "+10 min post DIS-hab" in their Fig. 2. That comparison establishes whether the dishabituating stimulus, dropping, restores response to the habituating stimulus, shaking, as demanded by Rankin et al. Gagliano et al. report "no difference between +6 h and +10 min post-DIS-hab in both environments, $p = 0.19$, $\chi^2 = 1.7$". Following Rankin et al.'s terminology, that means an absence of dishabituation.

What Gagliano et al. reported as dishabituation is, according to Rankin et al., a test of stimulus specificity. However, I argue that establishing stimulus specificity requires an addition to the experimental design not mentioned by Rankin et al., but included in Kenzer et al.'s (2013) study.

Gagliano et al. reported that when shaken plants were dropped, responding to that novel, dishabituating stimulus returned to original baseline levels: "Fig. 2; differences between +6 h and DIS-hab, $P < 0.0001$, $\chi^2 = 350$ for both environments". That result meets Rankin et al.'s definition of stimulus specificity: "To test for stimulus specificity/stimulus generalization, a second, novel stimulus is presented and a comparison is made between the changes in the responses to the habituated stimulus and the novel stimulus." That still leaves open the possibility that the dishabituating stimulus succeeds in restoring the response merely because the stimulus is more intense. Exhaustion of energy could produce the results reported by Gagliano et al. by raising response thresholds, such that a more intense stimulus is needed to elicit a strong response. The data that Gagliano et al. reported in Fig. 2 are entirely consistent with their shaking procedure being a more intense stimulus than their drop. They could have tested that possibility by counterbalancing which stimulus was the habituating stimulus and which the dishabituating stimulus, as Kenzer et al. (2013) did. Restored leaf folding when the stimulus changes, regardless of which stimulus serves as habituating and which as dishabituating stimulus, would have conclusively established stimulus specificity. Gagliano et al. did not include that counterbalancing in their experimental design. Thus, their data are not enough to support the second core claim of their paper that *Mimosa's* response exhibits stimulus specificity. Furthermore, that leaf folding decreases more quickly under low light is also consistent with exhaustion (Fig. 1).

Likewise, the data from Gagliano et al.'s short-term memory experiment, that plants kept in low lights folded their leaves less 6 days after training, are compatible with low-energy reserves causing a higher response threshold. There is even a problem with the one finding that appears, at first sight, to conflict with this interpretation: in the long-term memory experiment, plants transferred from high to low light 28 days before testing folded their leaves to the same extent as the group that experienced the opposite change. 28 days of low light may be expected to reduce leaf folding (increase leaf openness), as in the low-light group of Fig. 2, if depleted energy reserves are the cause of reduced leaf folding. The lack of a group difference in the long-term memory experiment may thus conflict with the exhaustion explanation. The problem with that argument is that leaf openness is, for five of the six timepoints, near maximal. Thus, a ceiling effect may explain the lack of a group difference. Possibly, an analysis of skew would address that. If the distributions of degree of leaf openness are not skewed, that would be inconsistent with a ceiling effect.

In summary, Gagliano et al. addressed an intriguing question, with an experimental design that could have provided an answer with small changes to analysis and experimental design. As it is, though, I argue that their conclusions go beyond their data.

Author contribution statement RB conceived, designed, and executed this study and wrote the manuscript. No other person is entitled to authorship.

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