On the Conditioning of Plants: A Review of Experimental Evidence

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Published online: 2 October 2018

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Abstract

Despite considerable research on the responses of plants to stimuli and a recent surge of interest in “plant intelligence,” few studies have been conducted on classical or respondent conditioning in plants. Studies of respondent conditioning in plants were reviewed, the majority of which used the sensitive plant (*Mimosa pudica*) as the subjects with seismonastic responses (leaflet-folding and leaf-drooping) as the unconditioned responses, and all of which used group designs. The reported results are mixed, with no replications of positive results. Issues have been noted with the methodology of these studies, including the lack of within-subject demonstrations, choice of putative conditioned stimuli, and potential unplanned interactions between subjects across experimental groups. Recommendations are made for addressing these issues in future research.

Keywords Conditioning · Methodology · *Mimosa* · Plant · Respondent

In his book *The Origin of Consciousness in the Breakdown of the Bicameral Mind*, Julian Jaynes (1976, pp. 6–7) describes an experiment in which he attempted to demonstrate classical or respondent conditioning in a plant. The subject was a sensitive plant (*Mimosa sp.*, presumably *M. pudica*), which has a seismonastic reaction, that is, the unconditioned responses of rapidly folding its leaflets and drooping its leaves following tactile stimulation (e.g., Armus, 1970; Holmes & Gruenberg, 1965). For the would-be conditioned stimulus, Jaynes (1976) used “an intense light” (p. 6). The specifics of the experiment are not given, but it is easy to imagine that Jaynes held a flashlight in one hand, turning it on for a second with the bulb close to the leaves, and then immediately touching the plant. As reported, after more than a thousand trials, the light did not elicit a response (p. 7). The experiment is notable for two reasons. First,

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there are few experiments in the published literature describing attempts at demonstrating respondent conditioning in plants, let alone successes. Indeed, if the usual result was negative, as was for Jaynes, then the lack of publications should not be surprising, especially if this result was expected or “obvious.” Second, and more important, the experiment may have been seriously flawed by not adequately accounting for the fact that the subject was a plant.

Jaynes’s experiment was clearly modeled on studies of respondent conditioning in animals (e.g., Pavlov, 1927, 1928). The light used for a putative conditioned stimulus would probably be adequate across a variety of animal species, which generally have eyes and are arranged to respond to visual stimuli, especially brief ones. Jaynes did not describe the temporal parameters of the stimuli, but if he was using animal studies as a model, then it is reasonable to assume that he used relatively brief stimuli, as would be appropriate for animals. Plants, however, generally operate on a slower timescale than animals (Trewavas, 2003) due to the way they are physically organized. Although a plant has leaves that use light to engage in photosynthesis, leaves are not arranged in such a way to capture visual stimuli the way eyes do. Although plants can respond to different qualities of light, including different wavelengths (e.g., Szechyńska-Hebda, Kruk, Górecka, Karpińska, & Karpiński, 2010), there are limits to any sensory system. If the stimulus was brief enough and the ambient light bright enough, it may be that the difference would not have been salient or even discriminable; what would be salient to a human might not be for a plant. Also, because light is biologically important to plants and may already elicit responses (e.g., Applewhite, 1972), it does not begin as a truly neutral stimulus; even if the plant can respond to the brief flash, there may be unexpected interactions and the results may be difficult to interpret.

The decentralized way that plants are physically organized relative to animals (Firm, 2004; Trewavas, 2003) may have also affected the results. As noted by Abramson and Chicas-Mosier (2016), the maximum response rate of *M. pudica* is about once every 15 min, limited by how quickly its leaves can unfold, and Holmes and Yost (1966) suggest an even lower rate. If Jaynes had run trials once every 15 min for 8 h a day, then to achieve the number of trials he claims would require running this experiment continuously for over a month. However, the lifespan of an *M. pudica* leaf is only about 4 weeks (Holmes & Yost, 1966)—just barely long enough to fit into this time frame. Barring this level of dedication, it is more likely that he regarded the whole plant as a single individual, distributing trials among multiple leaves and expecting the effects on one to transfer to the plant as a whole. If the plant at any time had 10 leaves and pairing on any 1 leaf was regarded as an independent trial, the whole study could have been completed in fewer than 4 days. However, given the decentralized organization of plant bodies, doing so would rest on an assumption that might generally apply to animals but would need to be proven with plants, and it is unclear what effect that might have on the (whole) subject’s ability to respond.

Finally, there is also the question of the choice of response, which may have been for the experimenter’s convenience. Leaflet-folding and leaf-drooping are relatively fast, discrete responses, much like the relatively fast, discrete responses often studied in animals (e.g., lever-pressing and key-pecking). This allows for a relatively straightforward adaptation of techniques from animal research to plants with little modification with regards to time. However, this might also be a problem. Plants are sessile beings, that is, they typically stay in place and are generally not arranged to make fast responses

on the same timescale as more motile animals such as humans, at least with regards to motion. Even when one response does have unusual parameters, it may not be shared by the same plant's other responses (e.g., *Mimosa* leaves do not turn towards light sources at a timescale noticeable to humans by casual observation). Even if Jaynes had gotten positive results, they might not generalize to other, more typical plant behaviors.

For the moment, let us accept (or at least entertain) that the actions of a plant count as behavior. This may be a controversial point for some (cf. Silvertown & Gordon, 1989), but it is arguably in line with Skinner's (1938, p. 6) early definition of behavior as "what an organism is *doing*" (emphasis in the original). Often with animal subjects, we equate this with movement; as Skinner elaborated on the above, behavior may be defined as "the *movement* of an organism or of its parts in a frame of reference provided by an organism itself or by various external objects or fields of force" (p. 6; emphasis added). Just as with animals, the actions of a plant may engage it with its environment, and often this is movement. For example, a plant's leaves may orient towards light, its roots grow down into soil or towards a water source, or its stems or tendrils move in such a way to secure the plant or move it over another plant or object. In animals, the behaviors of typical interest to behavior analysts historically have often involved the skeletal muscles and are presumably mediated by the nervous system (e.g., the triggering of mechanisms within an operant chamber by means of a beak or limbs). In contrast, plants have neither, but generally move by growth (e.g., Trewavas, 2003; Wood, 1953) or changes in internal fluid pressure (e.g., Bose, 1928). This makes many of the movements of plants more akin to the growth of tissues in animal bodies, such as growth in response to physical stresses or gravity (e.g., Brook et al., 2016), which is generally not treated as behavior. The obvious movements of *M. pudica* are of the latter type, controlled by the fluid content of special organs called pulvini, with movements occurring when the fluid pressure inside them changes (Allen, 1969; Bose, 1928; Volkov, Foster, Baker, & Markin, 2010). Not all mechanisms of plant movement are as well understood, such as the complex mechanisms involved for the Venus flytrap (*Dionaea muscipula*; Hodick & Sievers, 1989), though what is known is still quite different from those underlying the movements of animals. In addition to movements, plants may engage in other responses that may be less salient to humans, such as chemical signaling (e.g., Mulligan, Chory, & Ecker, 1997); the lack of any obvious motion does not change that this is also "doing," as well as part of a plant's way of interacting with its environment, and so it also may be taken as behavior. Some may argue that the differences in the forms of these actions or the underlying mechanisms might be reason to discount them as behavior. However, as behavior analysts, we are generally more interested in how something an organism does relates to events in its environment than the underlying mechanisms, and plants indeed do "something." For the purpose of this article, this objection is set aside for the moment, especially because even Skinner (1953, pp. 257–282) admitted some private events in humans as behavior, which may not involve skeletal muscles, but that may be understood as phenomena that are lawfully related to their environments.

There is an abundance of evidence that plants are not "mere" automatons, that is, they do not simply function and grow regardless of what is happening in their environments. Rather, just as is the case with animals, plants respond to a variety of stimuli involved in growth, collecting resources, and defense, as has been long demonstrated (e.g., Bose, 1906, 1928; Darwin, 1880) and continues to be demonstrated

up to the present (see Karban, 2008, for a review). In addition to the inanimate aspects of their environments, plants may respond to the presence of other plants, both their mere presence and chemical signals they release to each other (e.g., Engelberth, Alborn, Schmeltz, & Tumlinson, 2004; Farmer & Ryan, 1990; Mulligan et al., 1997; Paré & Tumlinson, 1999), much as animals may respond to stimuli created by other animals (e.g., Skinner, 1957). Likewise, the strength or amplitude of a response is not necessarily fixed, but may vary with exposure. For example, Holmes and Gruenberg (1965) demonstrated habituation of leaflet-folding and leaf-drooping in *M. pudica* to drops of water falling on the leaves at semiregular intervals. Notably the responses were specific to the stimulus presented (e.g., touching the leaf elicited the responses even after it habituated to the water) and the persistence of the habituation was limited, failing to occur the next day without being retrained. This phenomenon is robust and has been replicated elsewhere. For example, Applewhite (1972) exposed isolated *M. pudica* leaves to repeated mechanical stimulation (being dropped), which caused the leaflets to fold, and found systematic variations in how long it took to for them to unfold based on the interval between stimulations and the magnitude of the drop. In more recent research, Gagliano, Renton, Depczynski, and Mancuso (2014) used a similar dropping procedure with *M. pudica* to study the persistence of such changes over time, finding habituation when the subjects were tested again 28 days after the initial training. Whether or not this is “true” habituation has been questioned on the basis of the underlying mechanisms (Abramson & Chicas-Mosier, 2016), but the above studies demonstrate that plants may respond less to a stimulus with repeated exposure within certain parameters, just as has been observed with animals.

Although habituation is an important phenomenon in itself, in itself it does not expand an organism’s behavioral repertoire. In animal behavior, repertoires may be modified through stimuli acquiring new functions by association with stimuli that already have functions, or by responses changing topography and frequency depending on the surrounding antecedents and consequences, that is, respondent and operant conditioning. The study of these began in earnest with Pavlov (1927), who was known for pioneering studies of respondent conditioning, focusing on circumstances that altered the antecedents of reflexes. Skinner (1938) was strongly influenced by Pavlov but recognized that many responses did not fit this paradigm, and since then operant behavior has been an important focus of behavior analysis, including in understanding problem behavior in applied settings (e.g., Iwata, Dorsey, Slifer, Bauman, & Richman, 1994). Since then, these phenomena have been reliably produced and are well-studied in animals, and although the conceptualization of behavioral phenomena may be disputed, the phenomena themselves are accepted as fact.

In contrast, respondent and operant conditioning in plants are not regularly produced in laboratories (or at least recognized as such), and there are few published works that have specifically examined associative conditioning in plants, let alone claimed to have demonstrated it. One might reasonably question whether the phenomenon even exists. As was discussed with Jaynes (1976), not all researchers may have adequately accounted for the differences between animals and plants in the design of their experiments. The purpose of this article is to review the existing published experimental studies specifically about respondent conditioning in plants and to evaluate the strength of the claims. The necessary accommodations in the design to demonstrate

respondent conditioning in plants, assuming it is possible, are thus discussed, with recommendations for future research in the area.

Experimental Studies of Respondent Conditioning

The majority of the available studies on plant conditioning were published in *The Worm Runner's Digest* and its successor, the *Journal of Biological Psychology*, all of which use *M. pudica* as a subject. Despite considerable earlier work on plants, a lack of earlier attempts at demonstrating respondent conditioning was noted by Haney (1969), though he suggested that earlier researchers “may have been aware of the possibility.” A publication bias against plant conditioning studies may have been perceived at the time; as noted by Levy, Allen, Caton, and Holmes (1970), the *Journal of Biological Psychology* “was the only publication with the editorial courage to print anything so controversial,” and nearly 20 years later, Silvertown and Gordon (1989) noted that even speaking of plant “behavior” was still “unorthodox.”

Holmes and Gruenberg (1965): Negative Results

In the first of these studies, Holmes and Gruenberg (1965) were first concerned with demonstrating habituation, with a study of respondent conditioning briefly mentioned. The authors used electric shock as a US (unconditioned stimulus), attempting to use contact between the leaf and one of the electrodes (which was a saline-soaked wick made of cotton) as the CS (conditioned stimulus), presented 10 s before the US. The choice of shock as the US was due to “historical reasons,” based on Thompson and McConnell (1955), who used a shock-light pairing with flatworms. Although contact with the leaf as a CS was perhaps unavoidable because the device could not deliver shock from a distance, no rationale was made for this choice. The shock was successful at causing both leaves to droop and leaflets to fold. Details of the training procedure were not provided, but the authors report the would-be CS failed to elicit a response even after this being continued for 4 days. Despite the negative results, the authors state “we have by no means abandoned the idea of learning in the sensitive plant.”

Holmes and Yost (1966): Negative Results

This article was aimed at replicating the results of both the habituation and respondent-conditioning experiments described by Holmes and Gruenberg (1965), with similar results. The authors noted issues with the use of *M. pudica* as a subject, including the number of trials that could be run per day, which might be as few as two, depending on how long it took for the leaves to reopen. In addition, the leaves themselves have a life span of only about 4 weeks, with them responding less to stimuli as they get older. Because the use of electric shock seemed to be shortening the life span of the leaves, different stimuli which were less “injurious” were attempted. In this version, a specific leaf receiving brief touch with a camel-hair brush was used as the CS, and the whole plant being dropped 0.5 in. (1.27 cm) as the US, which elicited responding through the entire plant. After 2 weeks of receiving an average of seven trials per day, the authors

report no evidence of conditioning. The experiment notably included controls which received only the US or only the CS.

Haney (1969): Mixed Results

Again, using the leaflet-folding and leaf-drooping responses of *M. pudica*, Haney (1969) attempted to condition changes in illumination as the CS. The subjects were grown and kept in a controlled experimental chamber for the duration of the experiment, with all subjects housed together but divided into three groups. For group A, the CS was a light–dark transition of the overhead lighting. For group B, the CS was a dark–light transition, which occurred 15 min after the light–dark transition. The US for both groups A and B was tactile stimulation 1 min after the transition. The control group was housed with the other subjects and experienced the same changes in light but did not experience any tactile stimulation. During the experimental trials, the author attempted to capture the subjects' behavior by means of photographs taken every 15 s, which required illuminating the experimental chamber in which all the subjects were housed for 1 s. During test trials, the lights were merely turned off and on without the tactile stimulation. Haney reports that most of the responding occurred in group B (dark to light) but not in group A (light to dark) or control.

The study has some serious limitations, most notably that his choice of conditioned stimuli is not neutral. As Haney himself notes, the onset of darkness is also a US for leaflet-closing (“nyctritropic movement”) and may also make the plant less sensitive to light, whereas the onset of light opens the leaflets. One could thus argue that the lack of responding in group A (light to dark) was because the would-be CS inhibited the response. Likewise, the brief illuminations necessary for photography might have also affected the plants' behavior. No rationale was given for this choice of CS. Another serious problem is that all subjects were housed and tested together in close proximity to each other, and sharing the same light and dark cycles. As plants may release chemical signals to each other, there was a potential that plants in one condition were signaling plants in other conditions, thus altering the results. An additional procedural issue is how the US was delivered. Although not explicitly stated, as the US was delivered by hand, this would suggest that the US must have been delivered in succession to each plant, which means that the procedure was more variable than implied. Haney himself suggested that the few responses observed in group A and the control group were due to the experimenter accidentally contacting those plants while stimulating those in group B. If this is so, then, given that the plants were all housed closely to each other, the number of responses in group B might also have been inflated by accidental contact as well.

Levy et al. (1970): Negative (and Confusing) Results

This study was an explicit attempt to replicate the results of Haney (1969), recreating the B (dark to light) and control groups of the original. Levy et al. (1970) largely replicated Haney's procedure, the main differences being that there was no intermittent illumination during the dark periods for photography, and that the training versus test regimen was altered. The procedure described by Haney suggests training trials and testing trials being held on alternating days for 8 days (there is some ambiguity in the

description), whereas the procedure for Levy et al. is much more intense, with seven training trials and ending on a testing trial, repeated over 10 days. In the experimental group, no responses were observed during the testing trials, whereas multiple responses were recorded for the control group.

The authors were unable to provide a full explanation of these results. It is interesting that the authors report also having 200 additional plants that were not part of the study, but that were also kept with the experimental group and exposed to the same light–dark schedule, and that also were observed to respond at the same time as the control subjects. One possibility they raised was the difference in the illumination during the dark periods of the training session, during which Haney had intermittent illumination to do photography whereas Levy et al. did not. How this would produce the different results from a respondent-conditioning standpoint is unclear. As with Haney (1969), the presence of other plants in different groups housed in the same experimental chamber might have affected the results, possibly even magnified by such a large number together. A full accounting of the results of both Haney and Levy et al. would likely require replication with better controls.

Armus (1970): Positive (but Problematic) Results

Armus (1970) attempted to condition *M. pudica* with a light–dark transition as the CS; no rationale was given for this choice, though it may have been a choice of convenience as he noted that darkness is not a neutral stimulus. Plants were kept in pairs in housing booths, one in the experimental group, one in the control group. Among measures to control light going in and out of the individual housing booths were curtains on each booth. Both subjects would receive stimulation in the form of “striking the main stem” as the US; in the experimental group, this occurred 2 min after the transition, but in the control group it occurred about 30 min before. US presentations were strongly associated with the booth curtains being opened 3 min before and closed immediately after each US presentation; as a result, every opening of the curtains predicted the occurrence of the US half the time regardless of the condition. After the conditioning phase there was an extinction (testing) phase in which experimental subjects were run under the same procedure as the control subjects. Across phases, subjects on randomized days were tested by exposing them only to the light–dark transition without exposure to the US. During the conditioning phase, latency to leaflet-folding after the onset of darkness became consistently shorter for the experimental group than for the control group, increasing to about 100 s; during the extinction phase, the experimental group continued to have shorter latencies for several test trials. Armus interpreted these results as indicating the darkness has acquired a CS property eliciting leaflet-folding faster. During the study, no leaf-drooping was recorded during tests. Armus noted that darkness is, at least initially, a US for leaflet-folding but not for leaf-drooping, which might explain the difference.

The interpretation is not as simple as the author suggests. Aside from issues with the choice of CS (a change in illumination, which is not neutral), as with many of the previous experiments, this study also involves subjects that were housed together. Although this would ensure that, within the pairs, the subjects would receive the same pattern of illumination, it also necessarily means that the subjects could influence each other. Thus, the results may reflect the interactions between the pairs of subjects, not the

behavior of independent individuals, and this possibility would have to be ruled out by further experimentation.

Gagliano, Vyazovskiy, Borbély, Grimonprez, and Depczynski (2016): Positive Results

After 1970, no articles on respondent conditioning in plants appear to have been published until Gagliano et al. (2016). This corresponds with a recent increased interest in “plant intelligence,” with the behavior often being conceptualized in terms of cognitive psychology (e.g., Gagliano, 2014; Trewavas, 1999, 2003; cf. Abramson & Chicas-Mosier, 2016; Firm, 2004) or even neurology (e.g., Bose & Karmakar, 2003; Brenner et al., 2006; cf. Alpi et al., 2007). Aside from addressing a topic that appears to have been previously abandoned in the literature, Gagliano et al. break significantly from their predecessors in the choice of subjects and methodology. In this study, pea plants (*Pisum sativum*) were placed in a Y-maze, one arm of which lead to light. Although the *M. pudica* experiments used unconditioned stimuli that elicited leaflet-folding and leaf-drooping, this study used a stimulus known to elicit growth in a particular direction as a dependent variable, that is, phototropism, determined by growth towards a particular arm.

In the first experiment, subjects began with a period of 5 to 8 days in which they were on a cycle of light for 8 h and dark for 16 h. After this, they were randomly assigned to one of two groups, in both of which they only received light for only 1 h at a time three times a day as part of training trials, in the form of a blue LED light (which plants are known to grow towards, confirmed again in pilot data; see Christie, 2007). In one group (fan plus light), a putative neutral stimulus (air flow from a fan) was predictive of the light in the same arm, whereas in the second group (fan versus light), the same stimulus was predictive of light in the other arm. (This control eliminates the potential confound that the air flow was not a truly neutral stimulus, and additional pilot data showed no effect of air flow on direction of growth. No specific rationale was given for this choice of CS, though it is in a distinct modality from the US and demonstrably neutral.) In the initial training sessions, the plants were exposed to the would-be CS for 60 min before the light (US) came on in the relevant arm; the US and CS then overlapped for another 30 min before the CS was off and the US was on alone for an additional 30 min. This was followed by a 60-min intertrial interval in which neither the CS nor US was present, with a total of three trials per day. The position of the US was randomized for each trial. Following training, the subjects were tested by leaving them in the maze for an entire day. The subjects were again randomly assigned into two groups; those in the test group were exposed to the air flow at the same times of day they had previously been exposed as part of training, whereas those in the control group were exposed to neither stimulus. All subjects in the control group grew towards the arm in which they last experienced the light. By contrast, in the test groups the majority of the seedlings grew towards the arm where light would have been predicted by the air flow (62% when air flow was used as a putative CS+, 69% when it was used as a putative CS-).

In the second experiment, the effect of temperature on conditioning was investigated. The reason for this is because temperature systematically varies over the course of the day, being higher during daylight hours and decreasing at night. In this

experiment, the procedure (looking at air flow as a potential CS+ only) was replicated, with training occurring during different parts of the daily temperature cycle. When training occurred when the temperature was high (as in daylight), the same conditioning observed in the first experiment test condition was replicated (towards the CS); tests occurring when temperature was low (as at night) replicated the first experiment's control condition (growth towards where the light was last previously), whereas tests during a transition between the two temperature phrases were undifferentiated.

The study has a notable “ethological” focus in gauging hypotheses and procedures to the specifics of being plants. The long timescale used (2-h sessions with a 1-h intertrial interval) accommodates the plant's sensory and motor scales. The second experiment, investigating the effects of temperature as associated with particular times of day accommodates that plants, like animals, may not be equally able to respond and be conditioned at all times of the day. Just as rats and pigeons will probably not perform well when they are fatigued or asleep, the pea plants do not condition well with light as a US when other cues are related to it being twilight or night.

As with many of the studies described above, one potentially problematic methodological issue is the housing of subjects together. It is unclear how this might have affected the results, though one might suggest that chemical signaling between subjects might have decreased the effect size. One potential difference that might limit the effects of such cross-signaling is the number of groups and randomized aspects spread across subjects (fan plus light against fan versus light, randomization of side, test against control), which was larger than some of the other studies (e.g., experimental against control in Armus, 1970). These contrary pairs (fan and light, side) might have resulted in simultaneous signal releases with contrary effects on the subjects. To resolve the actual effects, exposure to other subjects might be included as an additional variable in future replications.

Limitations of Existing Studies and Directions for Future Research

Taken together, the results of the studies are mixed, ranging from negative (Holmes & Gruenberg, 1965; Holmes & Yost, 1966) to positive (Armus, 1970; Gagliano et al., 2016) to unclear and possibly with uncontrolled confounds (Haney, 1969; Levy et al., 1970). The results of Gagliano et al. (2016) are among the clearest, and, it is interesting to note, the only one of the studies not to use *M. pudica* for subjects. It is also one of the clearer ones experimentally because it does not use a CS that is not also a known or potential US. However, as of this writing, this study remains unreplicated. Given the lack of consistent results at this point (including the “paradoxical” results in Levy et al., 1970), the necessary conditions to produce respondent conditioning in plants are not well-understood, and some might argue there remain alternative explanations for the reported positive results. Given many behavioral phenomena can and are regularly demonstrated with animals in research, we are inclined to say they exist. At best we have a “maybe,” a few demonstrations, but more replications and extensions are needed, as well as better indications of the conditions under which respondent conditioning does and does not occur in plants. Given the inconsistent results, the reasons for this might be rooted in problems with

the methodology or research design. Future research should not only attempt to replicate the positive results but to improve on the designs to eliminate potential problems.

Perhaps one of the more fundamental issues is the choice of subject. Other than Gagliano et al. (2016), the above studies have used *M. pudica* for subjects. In basic research, behavior analysts have tended to work with rats (*Rattus norvegicus*) and pigeons (*Columba livia*), which are small, easy to care for, and whose responses can be generalized to others of interest (especially the behavior of humans). However, as stated above, leaflet-folding and leaf-drooping are not typical responses for a plant based the timescale on which they operate, nor is this movement based on growth. There is no guarantee that the lawful relationships discovered for leaflet-folding and leaf-drooping would generalize to other plant responses based on other mechanisms. The response in *P. sativum* investigated by Gagliano et al. (phototropism) is far more common and fundamental and, one could argue, a much better representative of plant behavior in general, both in terms of the underlying mechanism (growth) and timescale. Gagliano et al. used seedlings, and there are a number of seeds that might be readily sprouted. However, to establish the generality of results, more mature plants would also have to be used. In animal studies, specific breeds are often used, which limits the genetic variation of the subjects. Given the lack of centralization in the organization of plant bodies compared to those of animals, it may be possible to treat specific leaves, branches, or roots as individual “subjects” for purposes of a study, or even use isolated parts (cf. Applewhite, 1972). However, given the concerns about interactions between subjects, these might be especially problematic when the “subjects” are parts of the same organism. Another option, while controlling for the possible interactions between plants, would be to use multiple rooted cuttings from the same plant to create subjects that are genetically similar.

The choice of experimental design might also require modifications. All the reviewed studies utilize group designs, which pool individual responses. In contrast, single-subject designs have been more typical in behavior analysis (Cooper, Heron, & Heward, 2007, p. 163). Although there is nothing fundamentally wrong with group designs, the question is which design would work best for the research question. Given the high variability in behavior reported above, it may be that the behavior of some subjects is easier to condition than others; instances where conditioning could be demonstrated in some individual subjects may be masked by a lack of conditioning in others (cf. Trewavas, 2003). Aside from potential mathematical issues with group studies such as the necessity of having a sufficiently large sample size, there is the issue of housing and caring for all the subjects. In some experiments the subjects were described as being housed or tested together (Armus, 1970; Gagliano et al., 2016; Haney, 1969; Levy et al., 1970), which, given the limits on space and resources, may be a practical necessity given the number of subjects. Using single-subject designs would allow for meaningful demonstrations of causality using fewer subjects, which would be helpful where space is limited.

In addition, given that plants may release chemical signals to each other (e.g., Farmer & Ryan, 1990; Hlaváčková & Nauš, 2007), the results of such experiments may be affected by subjects interacting with each other, including across groups. Because such signals may not be detectable by researchers without special equipment, it is possible that inadvertent confounds may contaminate the results, as

especially may have been the case for Haney (1969) and Levy et al. (1970). Until it is firmly established that the US involved in a study does not elicit the release of chemical signals (or at least that they have no effect on other subjects), it should always be assumed that such signals may exist and measures taken accordingly. One option would be to house subjects separately and take measures to ensure limit exposure to external chemical signals, such as putting subjects in sealed containers, which would presumably block any such signals; however, some signals may be unknown to researchers and not be blocked by conventional means (cf. Gagliano & Renton, 2013). Given that responses to chemical signals released by other plants is part of a plant's behavior, another option would be to include exposure to such signals as a variable in behavioral research. Given the inconsistent and confusing results of the above citations, comparisons of exposed versus nonexposed plants may be necessary to fully understand account for such results. At this time, except when intersubject interactions are what is being systematically manipulated, plant subjects always should be housed and tested in isolation from each other to avoid potential confounds. Establishing adequate standards for controlling subjects' environments may require further research, and until this is done, researchers should be cautious in their designs.

Another important issue is the decentralized bodily organization of plants relative to animals. None of the reviewed studies directly addresses the issue, though Jaynes (1976) was the only one where it appears to have presented a problem. If behavioral phenomena are demonstrated, the question would then arise as to exactly what subset of the plant they apply relative to the subset involved in acquisition. For example, if habituation is demonstrated to sudden deceleration of the whole plant, can it be demonstrated to persist in an isolated part of the same plant? If a CS is trained for leaflet-folding for one leaf of an *M. pudica* plant, will that stimulus have the same effect for other leaves on the same plant that were not directly trained? If a *P. savitum* plant is shaped by differential reinforcement to grow towards an arbitrary stimulus, will a rooted cutting from that plant also grow towards that same arbitrary stimulus? If there is such a transfer, would the chemical signals that may confound other plant studies mediate the transfer, or are there other mechanisms? Such questions are important because they reflect an issue less commonly considered with animal subjects, namely the potential disunity of the individual. Presumably there are lawful relationships between what portion of a plant directly contacts the effective part of its environment and on what parts of that plant the effects can be demonstrated; for an experimental analysis of plant behavior to be complete and fully address the issues of plants, these relationships must be considered.

One potentially serious issue is dependent measures. In a typical behavior-analysis experiment with rats or pigeons, the subject is placed in an operant chamber or "Skinner box," which allows for the animal's behavior to be mechanically recorded (e.g., Skinner, 1956). This means that the behavior measured is relatively well-defined and free from a human observer's subjective bias. In contrast, plant behavior is typically measured by humans, who are potentially prone to measurement bias, especially when they are aware of the hypothesis. Some studies have taken measures to limit potential bias. For example, in Gagliano et al. (2016), efforts were made to have the observers checking for plant movements to be blind to the hypothesis. However, Gagliano et al. were exceptional in this regard. Levy et al. (1970) provided an

operational definition of the dependent measure by stating “Movement was scored if there was folding of leaflets or drooping of stems,” but none of the other studies involving *M. pudica* provided such a definition. Working with *P. savitum*, Gagliano et al. (2016) described the dependent measure as “the arm of the maze they [the seedlings] had grown into,” which is potentially ambiguous if the plant grows in anything but a straight line, though admittedly the lack of branching in pea seedlings severely limits this possibility.

How to fully address this issue remains unclear. Mechanically recorded dependent measures would be preferred to eliminate observer bias (cf. Abramson & Chicas-Mosier, 2016), as is in the case of operant chambers. However, this is relatively easy only when the subject is capable of making repeated movements that can be recorded by the same switch. For example, a rat is capable of physically pressing down on the same switch repeatedly, but this depends on the organization of its body. To have an *M. pudica* leaf likewise trigger a mechanism would be more difficult to achieve because of the lower pressure the leaf could exert on a switch and that the position of the switch would have to be adjusted as the plant grows. In addition, a rat can aim for the switch, whereas a *M. pudica* plant does not appear to have the senses nor mechanisms for movement to make this possible. A version of an operant chamber for *M. pudica* and similar plants would require the engineering of switches that are not only capable of detecting relatively delicate movements, but also could be repositioned as the plant changes and do not require the plant to aim. Although this could be done by having a camera looking at the plant and a computer program determining when a leaf has moved, a mechanical switch may be a better choice because cameras require light to work, which could lead to problems when access to light itself is an independent variable. Such a paradigm would not be viable for growth-based behavior, and in these cases a Y- or T-maze, similar to what is described in Gagliano et al. (2016), may be the best option. As discussed above, in the absence of a mechanical measure, strict adherence to an operational definition should be the norm, as well as interobserver agreement measures as is common in applied work. This may also to some degree be addressed by subject selection, such as seedlings with little or no branching; however, as the research is extended to more mature plants, this may not be practical.

Another issue that needs to be addressed is the timescale involved. As discussed, plants generally are organized to respond much more slowly than motile animals; lacking the kinds of sensory organs found in animals, brief stimuli that would be salient to animals may not necessarily be salient to plants. Gagliano et al. (2016) is notable for having fairly long, overlapping presentations of the CS and US. In contrast, *M. pudica* studies generally used much shorter durations. Although there may be some particular individuals which can respond to brief stimuli, especially intense ones, the likelihood of getting positive results may improve if the plant's timescale is accommodated. It is notable that even though the majority of behavioral studies involved *M. pudica*'s leaflet-folding and leaf-drooping responses (which is unusually fast for a plant), brief stimuli did not reliably produce conditioning. Future studies will need to better accommodate plants as subjects by extending the timescales as Gagliano et al. (2016) did, which means that typical studies will have sessions that last for hours. This will be easier for researchers if it can be automated, to decrease the direct effort involved and to ensure the procedure

is implemented consistently, both of which could become issues as session length increases. To study the movement of *M. pudica* leaves and similar behaviors, once the proper switches have been created to detect these movements, it should be relatively simple to automate a plant version of an operant chamber. Some innovation may be required to find ways to present stimuli reliably through automation, such as using a piston or puffs of air to trigger responses, but such setups would largely be extensions of existing technology. For maze studies, as described above, mechanical collection of data may be less practical, though with automated presentation of stimuli, human involvement could be limited to the initial setup and final measurement at the ends of sessions.

Aside from the timescale, the most important issue to be addressed in future research is the choice of conditioned stimuli. Illumination, or rather changes in illumination, was used in most experiments as the CS, but this is a problematic choice as changes in illumination are biologically important to plants and may already be unconditioned stimuli. Furthermore, the effects of some eliciting stimuli may be complicated, including light. For example, Applewhite and Gardner (1971) noted that, although light is usually a US for leaflet-opening in *M. pudica*, a dark–light transition after an extended period of dark elicits leaf-drooping. The choice to use illumination changes may be influenced by the relative convenience of using it, and the use of similar stimuli in animal studies. However, by using a CS that is already a US, possibly for the same response being conditioned, there may be undesirable interactions between the two. In contrast, Gagliano et al. (2016) used conditioned stimuli with no inherent relationship to the response of interest. Given that some stimuli may condition more readily to particular stimuli in animals (e.g., Garcia & Koelling, 1966), it may be profitable to investigate a variety of stimuli.

The above studies have handled conditioning in plants as if this were respondent conditioning, and this may yet be the case, but not necessarily so. In Gagliano et al. (2016), the “CS” and “US” occurred over extended periods. For animals, which live on a much more rapid timescale, even with an overlap, there is a clear sense of which stimulus is “first,” but with a plant, with both stimuli occurring simultaneously over an extended period, the distinction between antecedent and consequence may become blurred. An alternate interpretation of the experiments would be that the putative CS was really a discriminative stimulus, and the putative US was really a reinforcer, that is, the plant’s growth in a particular direction was reinforced by access to the blue light. This would especially explain why the subjects tended to grow towards the arm where they last experienced the light, that is, where they were last (putatively) reinforced. The changes in temperature in the second experiment accordingly could be interpreted as motivating operations affecting the value of the light as a reinforcer. Hence, what has been conceptualized as respondent conditioning might really be operant conditioning. This interpretation, of course, would need to be confirmed, but the test might be relatively straightforward in principle, analogous to demonstrating reinforcement of animal behavior. For example, a plant might be grown in a chamber receiving low but sufficient diffuse light to sustain it, with the direction of its growth monitored. Presumably the plant will not grow in a perfectly straight line (cf. Darwin, 1880), and from this variation, growth in a particular direction might be selected with access to a much stronger and presumably more highly preferred light. (This might be checked

simply by growing a subject in a Y-maze similar to the one in Gagliano et al., 2016, with the stronger and more diffuse lights in different arms; the subject would presumably grow towards the “preferred” option.) Given that the timescale involved would be much slower than in an animal experiment, some modification of the procedure would be required. It would be necessary to determine the smallest unit of time during which growth in a particular direction could be measured, after which this interval could be applied in the observation of the subject, which is checked regularly at the end of this duration. If, at the end of an interval, the subject has grown in an arbitrary desired direction, then the more intense light would be turned or remain on; if at the end of an interval the plant is growing in another direction, then the light would be turned off again. If the intense light is not a reinforcer, then a zigzag pattern of growth would be expected, with growth veering towards the light whenever it is turned on, but much more variable when it is off. However, if the intense light is a reinforcer, then the plant would grow towards the direction in which it is consistently reinforced, regardless of wherever the light is placed in the chamber. Such an analysis does not lend itself well to the above experiments with *M. pudica*, both for the design of the experiments (both stimuli and responses were much shorter and discrete) and the nature of the response itself (different underlying mechanisms), and it is unclear what would be a reinforcer for leaflet-folding and leaf-drooping. Given that typically studied animals do both respondent and operant behaviors, it may be that the rapid pulvinus-based behaviors of *M. pudica* and other plants are fundamentally functionally different from growth-based plant behavior.

Finally, as mentioned earlier, some plant behavior arguably is similar to growth in animal bodies not typically included as behavior. Assuming future research does confirm that plants are capable of having their “behavior” conditioned similarly to that of animals, what impact would this have on the definition of behavior? Perhaps not much. Animal bodies are already known to engage in actions independent of the nervous system, such as immune responses, and these may change as a result of what pathogens the immune system is exposed to (or in lay terms, the immune system “learns”). This alone has not been enough to consolidate immunology under psychology. However, such discoveries might be cause for reconsideration, just as it has in other fields. For example, the body known as Pluto was considered a “planet” since its discovery in 1930, only to be reclassified as a “dwarf planet” following the discovery of a trans-Neptunian object that appeared to be larger, aptly named Eris after the Greek goddess of discord (Ekers, 2006). The term “planet” had not been well defined before, and the discovery of Eris forced astronomers to reconsider what the term meant, or rather what it should mean. Likewise, when talking about “behavior,” our ideas and definitions are generally adequate for the exemplars we deal with. For example, applied behavior analysts might accept clients to treat self-injurious behavior or train life skills, but there are probably few attempting to condition immune responses. However, there may be cases that require those definitions be reconsidered. There is already evidence that some drug tolerance may have an underlying basis in respondent conditioning (e.g., Carlton & Wolgin, 1971), so other bodily responses acting like more “traditional” behavior cannot be ruled out a priori. The question of whether such a bodily action, such as skeletal or muscular growth, can even be conditioned, of course, remains an empirical question.

Acknowledgements The author would like to thank Daniel Kueh and Nancy Ann Adelman for their help in obtaining relevant articles and support in the preparation of this manuscript, as well as Kelly Kates McElrath and Tim Van Tillburg for their comments.

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