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Habituation and Dishabituation to a Vibrational Stimulus by a Millipede, *Orthoporus texicolens*

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ABSTRACT

The primary defense mechanism of the North American millipede, *Orthoporus texicolens*, is coiling. We studied habituation, a form of non-associative learning, by observing coiling in response to a vibrational stimulus. Coiling declined in successive trials, indicating that habituation occurred. Dishabituation was then studied by not presenting the vibrational stimulus for intervals ranging from 4-14 minutes. The response of coiling did begin to reappear, showing evidence of dishabituation. Of the experimental millipedes, 75% habituated to vibrational stimuli, and of these millipedes, 100% dishabituated due to the lengthening time span between each vibrational period. Our data indicate that millipedes are able to habituate to a stimulus and then dishabituate to the stimulus if the time intervals between stimulus presentation are lengthened.

INTRODUCTION

Habituation is a type of non-associative learning when an organism learns not to respond to an irrelevant stimulus that initially elicited a response (Muller and Hildebrandt, 2002). Habituation is important for organisms receiving multiple sensory cues from their environment because it allows for stimulus filtering (Gray, 2005), thus enabling organisms to learn to react only to biologically relevant environmental stimuli (Mankin et al., 1999; Best et al., 2005). For example, avoiding predation becomes more likely by focusing on stimuli produced by predators; less threatening stimuli do not trigger a response, thus reducing the amount of time expended on irrelevant stimuli (Gray, 2005). In order for habituation to occur, the organism must not be food deprived or unhealthy (Braun and Bicker, 1992), and the stimulus must be presented repeatedly (Wyttenbach and Hoy, 1997). Often habituation is considered to be short term (Engel and Hoy, 1999) and is considered a simple form of learning (Bristol and Carew, 2005).

Various invertebrates such as honeybees (Braun and Bicker, 1992), crickets (Engle and Hoy 1999), leeches (Burrell et al., 2001), flatworms (Koopowitz, 1974), and mollusks (Bristol and Carew, 2005) have been shown to habituate to irrelevant stimuli. Once a habituated organism comes in contact with a different form of the stimulus, it may respond as if the stimulus is novel. This new response is known as dishabituation (Burrell et al., 2001). It is not adaptive for habituation to occur without the mechanism for dishabituation (Krasne and Glanzman, 1995).

Habituation in millipedes has not previously been studied. The purpose of the study was to examine whether millipedes habituate to a vibrational stimulus and then to test for dishabituation.

METHODS

The organisms tested were North American millipedes, *Orthoporus texicolens*. Each millipede was housed separately in a holding facility (11.0 cm diameter x 10.0 cm h) containing moist topsoil, and food and water were provided *Ad libitum*.

Millipedes avoid predators by using two different defense mechanisms - excreting a noxious substance or coiling into a ball with the head tightly covered. Since not all species use chemical secretions to deter predators (Arab et al., 2003), we used millipede coiling to indicate defensive behavior. We used the smallest possible frequency of vibration to illicit a response from the millipedes since weaker stimuli require decreased time for habituation to occur compared to stronger stimuli (Koopowitz, 1974).

We used 40 millipedes randomly separated into 20 control millipedes and 20 experimental millipedes. The experimental millipedes received the vibrational stimuli repeatedly to test for habituation, while the control millipedes only received two vibrational stimuli separated by a 78 minute time interval.

Each millipede was positioned on top of a platform that was directly in contact with a vibrator (an Elenco GF- 8026 function generator hooked to Pasco Scientific SF- 9324 mechanical vibrator). In order to examine habituation, experimental millipedes were exposed to a vibrational stimulus of 37 hertz for 5 seconds. We conducted 40 trials on each millipede with 2 minute intervals between each vibration. Control millipedes were only exposed to the vibrational stimulus twice for 5 seconds, with the stimuli separated by a 78 minute time interval. Presence or absence of millipede coiling was recorded for all trials.

We then exposed all experimental and control millipedes to the same conditions to examine possible dishabituation responses in each of the two groups. We exposed millipedes to additional vibrations after trial number 40, but instead of a 2 minute interval between vibrations, one stimulus was skipped, leaving a 4 minutes interval until the next stimulus. Next, two stimuli were skipped leaving a 6 minute interval. This pattern of skipping stimulation continued until 6 vibration periods were skipped, leaving an interval of 14 minutes between stimuli. Similar methodology of altering interval lengths between the previously used stimulus was employed to test for dishabituation of acoustic startle stimuli in mice (Sasaki et al., 2001)

We used two regression analyses to determine the change in proportion of millipedes that responded over time. One analysis represents presence of the coiling response of millipedes over time and the other represents absence of the coiling response in millipedes over time.

RESULTS

Since the research consisted of two different experiments (habituation and dishabituation), two sets of data were collected. At the beginning of the habituation experiment, 95% of the experimental millipedes coiled; whereas, after 40 trials, significantly less (25%) continued to coil (*P* = 2.15E-22) (Fig. 1). In contrast, 95% of the control millipedes initially coiled, and after our interval of 78 minutes, 90% still responded (Fig. 1).

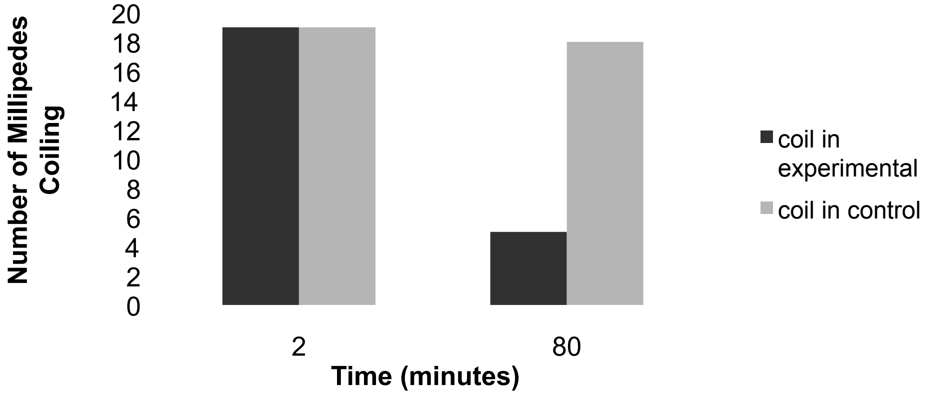

Figure 1. Habituation study: Response of experimental millipedes (subjected to 40 vibrations at 2 minute intervals) and control millipedes (subjected to only 2 vibrations with an 78 minute interval) during the first and last trials.

The dishabituation experiment involved 15 habituated experimental millipedes that no longer responded to the vibrations and 15 control millipedes. During the first trial of the dishabituation research, none of the experimental millipedes responded to the vibrational stimulus (Fig. 2). However, significantly more (100%) of the experimental millipedes responded during the last trial conducted 54 minutes later (*P* = 0.000439) (Fig. 2). In contrast, 93% of the control millipedes responded to the first trial vibrational stimulus, and 100% still responded after the 54-minute interval (*P* = 0.140) (Fig. 2).

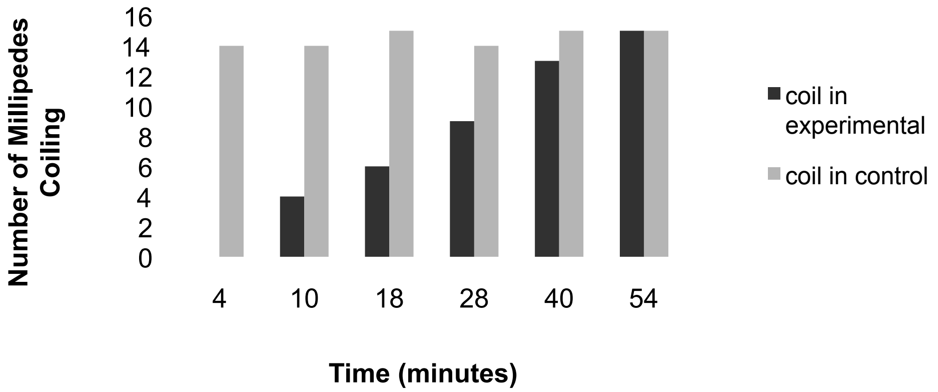

Figure 2. Dishabituation study: 15 habituated experimental and 15 control millipedes' reactions to the vibrational stimulus. Each time interval between the vibration stimuli was 2 minutes longer than the previous vibration causing longer time periods between vibrations to occur in successive trials.

DISCUSSION

Experimental millipedes exposed to repeated vibrations did habituate while control millipedes, exposed to only two vibrations, did not. Initially, the response to the vibrational stimulus was coiling, but toward the end of the trials, experimental millipedes no longer responded while control millipedes continued to respond. Habituation occurs in response to a repeated irrelevant stimulus (May and Hoy, 1991). Millipedes initially responded via coiling because the stimulus was novel. After repeated trials, millipedes learned not to respond to the irrelevant stimulus. Thus, non-associative learning was demonstrated for the first time in a species of millipede. There is additional support for habituation in uniramia since, upon exposure to a repeated electrode stimulus, some insects also habituated (Braun and Bicker, 1992; Engle and Hoy, 1999; Friedel, 1999). For example, locusts undergo habituation by decreasing startle responses to repeated vibrational stimuli that are not associated with reward or punishment (Friedel, 1999).

Dishabituation also occurred as indicated by the experimental millipedes response to the vibration as time between trials increased. The longer the time span between vibrational stimuli, the more novel the stimulus seemed; therefore, habituation was no longer intact (Wyttenbach and Hoy, 1997). Since the control millipedes had not undergone habituation, their response rate remained constant throughout.

Our research demonstrates the presence of non-associative learning (habituation) and subsequent dishabituation in an invertebrate. This is the first study of habituation and dishabituation in millipedes. Further research should explore the role of associative learning in these invertebrates.

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