Volume 88, No. 1, September 2011

ISSN 0376-6357
BPRODA 88 (1) 1–66 (2011)

ELSEVIER

Behavioural Processes

Editors: F. Cézilly & C.D.L.Wynne

Auditory stimulation dishabituates anti-predator escape behavior in hermit crabs (*Coenobita clypeatus*)

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ARTICLE INFO

ABSTRACT

Responses to innocuous stimuli often habituate with repeated stimulation, but the mechanisms involved in dishabituation are less well studied. Chan et al. (2010b) found that hermit crabs were quicker to perform an anti-predator withdrawal response in the presence of a short-duration white noise relative to a longer noise stimulus. In two experiments, we examined whether this effect could be explicable in terms of a non-associative learning process. We delivered repeated presentations of a simulated visual predator to hermit crabs, which initially caused the crabs to withdraw into their shells. After a number of trials, the visual stimulus lost the ability to elicit the withdrawal response. We then presented the crabs with an auditory stimulus prior to an additional presentation of the visual predator. In Experiment 1, the presentation of a 10-s, 89-dB SPL noise produced no significant dishabituation of the response. In Experiment 2 we increased the duration (50 s) and intensity (95 dB) of the noise, and found that the crabs recovered their withdrawal response to the visual predator. This finding illustrates dishabituation of an antipredator response and suggests two distinct processes—distraction and sensitization—are influenced by the same stimulus parameters, and interact to modulate the strength of the anti-predator response.

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1. Introduction

Responses to innocuous stimuli often habituate with repeated stimulation (Thompson and Spencer, 1966; Groves and Thompson, 1970; Rankin et al., 2009). Habituation has been shown in a variety of invertebrates, such as *Aplysia* (e.g., Castellucci et al., 1970; Hawkins et al., 2006), *Drosophila* (e.g., Corfas and Dudai, 1989), and crayfish (Krasne, 1969; Krasne and Bryan, 1973; Shirinyan et al., 2005), as well as in a number of distinct modes of behavior, including the startle reflex (Halberstadt and Geyer, 2009), and anti-predator behavior (Vowles and Prewitt, 1971; Krasne and Teshiba, 1995; Tomsic et al., 2009). A stimulus that initially generates anti-predator escape behavior may be habituated through repeated presentations. For example, Krasne and Teshiba (1995) found that crayfish habituated to the presentation of a mild shock that initially generated a tail-flip escape reflex.

Sensitization is another major type of non-associative learning process. While habituation is characterized by a reduced responsiveness to a particular stimulus over repeated trials, sensitization is typified by increased responsiveness not only to the recurring stimulus but more broadly to other stimuli as well (Thompson and Spencer, 1966). A crucial difference that determines whether habituation or sensitization occurs is the intensity of the stimulus. Habituation is more rapid with low-intensity stimulation (e.g., Groves et al., 1968); with intense or noxious stimuli, sensitization is likely to occur first, followed by habituation (e.g., Davis et al., 1982). The presentation of a novel, arousing (i.e., sensitizing) stimulus can cause the recovery of a previously habituated response; this effect is known as dishabituation (Thompson and Spencer, 1966; Castellucci and Kandel, 1976). Though recent evidence has demonstrated that dishabituation and sensitization rely on distinct neurochemical mechanisms (e.g., Antonov et al., 2010; Byrne and Kandel, 1996; Marcus et al., 1988; Rankin and Carew, 1988), the two effects are quite similar on a behavioral level. Indeed, stimuli capable of producing sensitization often will also dishabituate previously habituated responses (e.g., Carew et al., 1971; Groves and Thompson, 1970; Thompson and Spencer, 1966).

In a recent experiment, Chan et al. (2010b, Experiment 2) examined the effect of extraneous noise on hermit crab withdrawal behavior. An approaching simulated visual predator (i.e., a hawk) elicited a protective response (withdrawing into the shell) in the crabs (see also Chan et al., 2010a). In one condition, a white noise was presented alone for 10 s, followed immediately by the compound presentation of the noise with the visual predator; in the other, the white noise was presented alone for 90 s prior to presen-
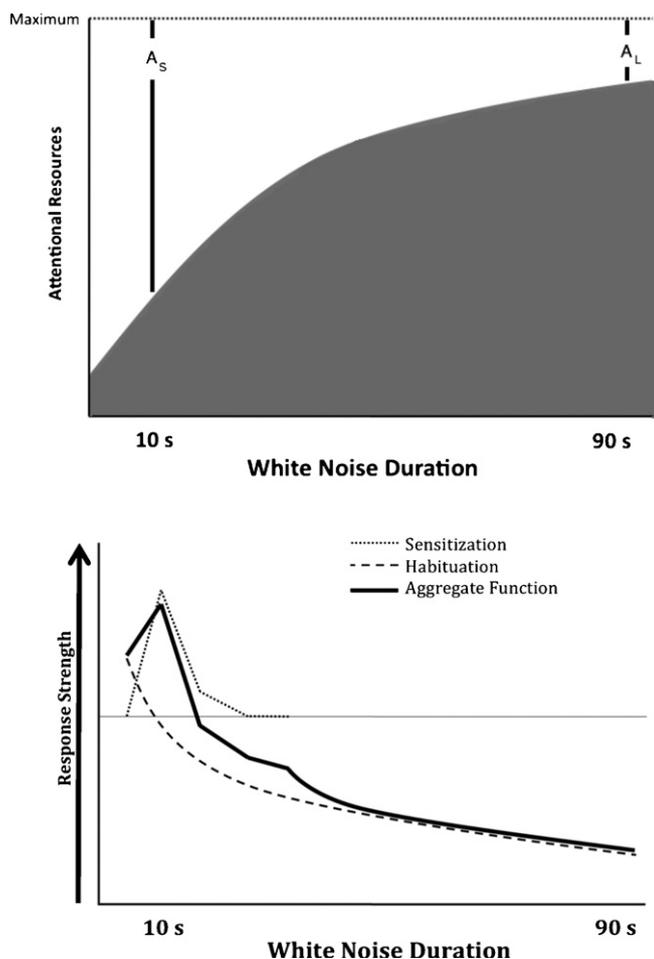
Fig. 1. Top panel: Potential attentional mechanism for the results of Chan et al. (2010b). The grey region indicates attention allocated to the auditory stimulus over presentation time. The white region indicates resources available to attend to other stimuli (e.g., the visual predator). A$_S$ indicates resources available for the short-duration stimulus trials, while A$_L$ indicates available resources for the long-duration trials. Bottom panel: Potential learning mechanisms behind the results of Chan et al. (2010b). The dashed lines indicate the two non-associative learning processes, while the thick solid line indicates behavioral strength as a sum of these two processes. Redrawn from Colombo et al. (1997).

tation of the compound stimulus. The 90-s white noise produced a higher latency to hide than the 10-s noise. There are two possible explanations for this difference. The long-duration stimulus may have been more distracting than the short-duration stimulus, thereby attenuating the withdrawal response (see Fig. 1, top panel); alternatively, the 10-s white noise could have sensitized the animals, thereby causing them to be more reactive and quicker to respond to the visual predator. This latter scenario suggests that subjects may have habituated by the end of the 90-s white noise, and thereby their reactivity to the visual cue would have returned to baseline levels (cf. Bashinski et al., 1985; Colombo et al., 1997; see Fig. 1, bottom panel). Here, we report results from two experiments that were performed to clarify the results of Chan et al. (2010b). We discuss the results in terms of the interaction between non-associative learning and attentional processes.

2. Experiment 1

2.1. Introduction

Chan et al. (2010b) found that hermit crabs' escape response to a simulated predator was more rapid when it was presented
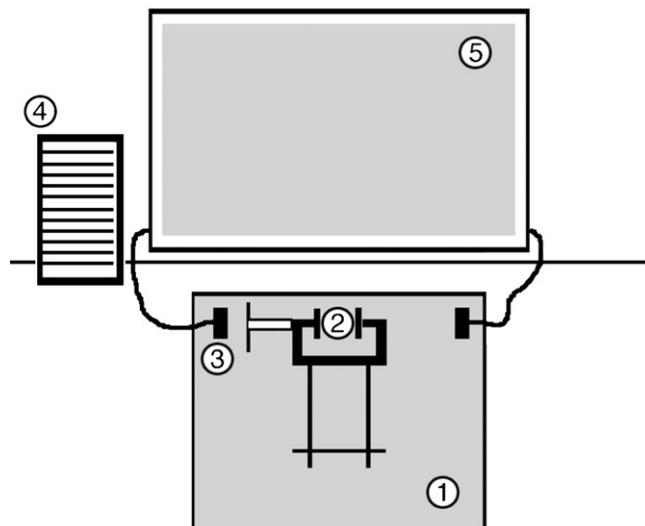
Fig. 2. A schematic of our experimental set up. (1) The automated withdrawal detector, (2) the crab holder (modified C-clamp), (3) the photobeam sensors (one on each side of the clamp; one emitter and receiver), (4) the Sony speaker, and (5) the LCD monitor. Redrawn from Chan et al. (2010b).

following a 10-s white noise stimulus than after a 90-s white noise. Because these two stimuli were presented together, it is not possible to properly distinguish between these hypotheses (i.e., distraction versus non-associative learning). In this experiment, we investigated whether a preceding extraneous 10-s noise, presented alone, could produce recovery of a habituated escape response. If the noise is indeed sensitizing at short durations, we should observe recovery of the escape reflex elicited by the simulated predator (i.e., dishabituation) following presentation of the 10-s noise. Alternatively, if the noise is not sensitizing, and is merely more distracting at long intervals, then no recovery of anti-predator behavior would be predicted following the 10-s noise.

2.2. Method

2.2.1. Subjects

Twenty Caribbean hermit crabs (*Coenobita clypeatus*) were obtained from an Internet source (live-hermitcrabs.com). The aperture length for the crabs' shells ranged from approximately 3–4 cm. Crabs were housed in groups of 5 in clear plastic tubs (approximately 50 cm × 25 cm × 25 cm) with sponges, coconut fiber substrate (Zoo Med Eco Earth), and plastic covers. The atmosphere in the tubs was maintained between 50 and 75% humidity. Crabs subsisted on a variety of dried fruit, hermit crab meal (Tetrafauna) and water. The subjects were identified by one of five colors of non-toxic OPI nail enamel painted on their major claw. We maintained the crabs on a 14 h/10 h day–night schedule; experimental procedures were conducted during the light portion of the cycle.

2.2.2. Apparatus

Fig. 2 shows a schematic of the experimental apparatus. The experiment was conducted in a 2.5 × 1.5-m soundproof room. The experimental setting consisted of one speaker (Sony SRS 77G) adjacent to a 17-in. (43-cm) Dell LCD monitor. The speaker provided auditory white noise at a fixed 89 dB sound pressure level (SPL) when measured at 0.30 m (the distance between the speaker and the subject) with a RadioShack sound meter (CAT 33-2055). We used the LCD monitor to display a visual stimulus, a wingspread hawk (Fig. 3) that started as a single pixel at the top and center of the screen, and then expanded and descended at a constant rate for 15 s

Fig. 3. The simulated visual predator. It began as a small point at the top of the screen and expanded as it descended over the course of the trial.

until it reached a maximum size of screen width (approximately 30 cm or 900 pixels wide) at the bottom of the screen.

We constructed and used an automated withdrawal detector (AWD), which was located approximately 10 cm in front of the LCD monitor (see Chan et al., 2010b). The AWD had two 20-cm sliding metal levers elevated 5 cm above the ground at its base, with an adjustable C-clamp attached to the same end on both levers. The levers attached to a 20 cm × 20 cm wooden platform and allowed us to adjust the clamp's position to each crab's shell size by enabling both vertical (to raise or lower the crab) and horizontal (to move the crab closer or further from the monitor) adjustment. We positioned an infrared photobeam emitter and detector such that an infrared beam ran parallel to the clamp's spine and over the crab's shell aperture. We designed its position so that, when a crab emerged while in the clamp of the AWD, its legs disrupted the beam. A computer recorded whether a crab performed an escape response during the experimental trial.

2.2.3. Procedure

We began the experimental session by putting an individual crab on the back of its shell into the AWD; pilot study indicated that this positioning ensured a reliable withdrawal response to a simulated predator, as well as a rapid emergence from the shell after hiding. A trial began when the crab emerged from its shell. Each trial commenced with a 60-s delay, followed by the presentation of the visual stimulus. The crab received repeated presentations of the visual stimulus until a trial in which it failed to withdraw into its shell. Following this trial, we presented the crab with 45 s of silence, followed by a 10-s presentation of the white noise. Five seconds after the termination of the noise, we presented the visual predator again.

2.3. Results and discussion

Two crabs escaped from the AWD during the experimental session and 5 crabs did not respond to the visual stimulus on the first trial[1]—we eliminated these crabs from analysis. The remaining

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[1] A reviewer raised concerns about the high number of crabs that failed to respond to the visual predator on the first trial of the session. We can only speculate as to the
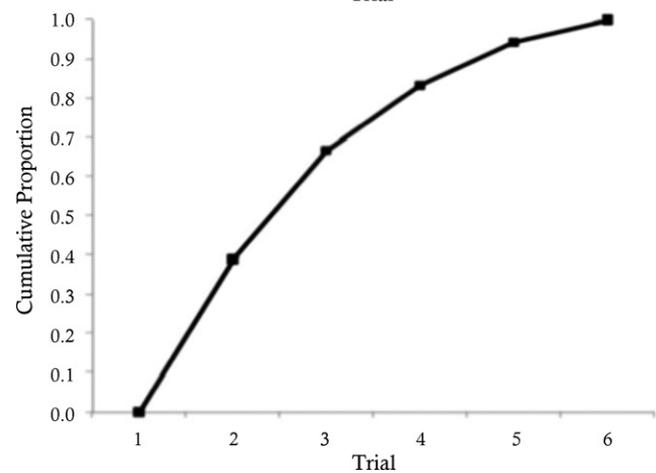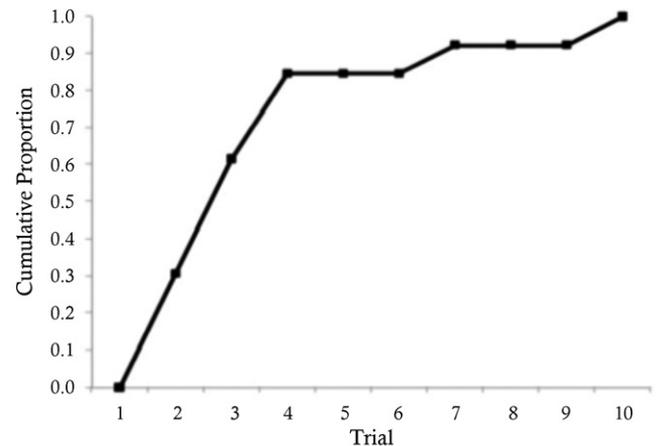
Fig. 4. Cumulative proportion of crabs failing to withdraw to the presentation of a predatory stimulus (i.e., habituation of the anti-predator escape response) over trials in Experiments 1 (top panel) and 2 (bottom panel).

13 crabs all stopped responding to the visual stimulus following a maximum of 10 exposures (Fig. 4A). On the trial following the auditory stimulus presentation, two of 13 crabs recovered their escape response to the hawk stimulus, which was not different than an expected baseline recovery of zero crabs ($p = 0.48$, Fisher's exact test).

These data reveal a failure of a 10-s white noise to produce dishabituation of the crabs' withdrawal response. This outcome suggests that the relative behavioral deficit produced by the long-duration noise reported by Chan et al. (2010b) was due to an attentional process in which the 90-s noise was more distracting than a 10-s noise, and not to the dynamics of non-associative learning processes. This finding does not, however, preclude the possibility that a sensitization process can play a role in dishabituating the crabs' withdrawal response.

3. Experiment 2

3.1. Introduction

In this experiment, we increased the intensity of the white noise stimulus from 89 dB SPL to 95 dB SPL—a doubling of auditory

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reasons why these five crabs initially failed to withdraw. We know from studies of other species of hermit crabs (e.g., Briffa and Twyman, 2011) and from recent work in our lab (Watanabe, under review) that there are repeatable individual differences in initial risk assessment. Alternatively, these five crabs may have been directing their visual attention at a location other than the monitor when the hawk was presented.

amplitude—as well as increased the duration to 50 s. Louder auditory stimuli have been shown to have a greater sensitizing effect (e.g., Davis, 1974). With the use of the more intense stimulus, we hypothesized that crabs may recover their habituated withdrawal response.

3.2. Method

3.2.1. Subjects and apparatus

The subjects and apparatus were the same as used in Experiment 1.

3.2.2. Procedure

The second experimental procedure was similar to the first, with three important differences. First, we increased the duration of the auditory stimulus from 10 s to 50 s. Second, we presented the stimulus at 95 dB SPL instead of 89 dB SPL. Third, we used a between-groups design. Following habituation, the intertrial interval (ITI) for Group Noise ($n = 10$) consisted of 5 s of silence, followed by the acoustic stimulus, which was followed by an additional 5 s of silence; Group Silence ($n = 10$) received 60 s of silence during the ITI. The visual stimulus was displayed again on the computer monitor immediately after the ITI.

3.3. Results and discussion

One crab in the control group failed to respond on the first trial of the visual stimulus, and was therefore excluded from the data analysis (final $n = 9$). All other crabs habituated to the visual stimulus in a maximum of six trials (Fig. 4B). The two groups of crabs did not differ in the number of trials to habituate, $t(17) = 0.094$, $p = 0.93$. At test, 8 out of 10 crabs in the Noise group recovered their escape response. In contrast, only one crab (out of 9) in the Silence group recovered the withdrawal response. This difference was significant ($p = 0.006$, Fisher's exact test). This indicates that the noise treatment was effective in recovering the crabs' anti-predator response.

4. General discussion

These experiments found that increasing the intensity and duration of a broadband auditory stimulus increased its effectiveness to dishabituate the withdrawal reflex evoked by a simulated visual predator in a terrestrial hermit crab. These findings are consistent with the results of earlier studies of dishabituation (e.g., Groves and Thompson, 1970; Davis, 1974; Pitman et al., 1990).

The experiments by Chan et al. (2010b), however, revealed decreased responsiveness to a simulated visual predator if it was accompanied by louder or longer white noise than if it was accompanied by a shorter or quieter white noise. If louder and longer white noise produced greater dishabituation in our current experiments, then why did they not similarly increase responsiveness in the experiments of Chan et al.?

We suggest that Chan et al. (2010b) observed decreased responsiveness because the white noise was present *during* the presentation of the visual stimulus; this shows that noise interferes with an *attentional* process. Nervous systems have a finite ability to process environmental information (Dukas, 2004). Distraction (i.e., attentional capture) is the involuntary allocation of attention to a salient stimulus. Thus, when the white noise is present, it draws some of the animal's limited attentional resources, resulting in impaired responsiveness to the simulated predator (see Fig. 1, top panel). By contrast, in the experiments reported in the current paper, we studied a *learning* process. We found that more-intense stimuli produce greater dishabituation. These stimuli are likely more salient as well, and therefore would command more of

an organism's attention when presented in compound with other stimuli, thereby reducing responsiveness to these stimuli (e.g., a simulated visual predator).

In conjunction with prior work with terrestrial hermit crabs (Chan et al., 2010a,b), we conclude that both distraction and dishabituation are likely modulated by the same stimulus parameters. That is, increases in the magnitude of the white noise increased distraction and facilitated dishabituation of crabs' escape response to a visual predator. The time course of the distraction effect appears to be limited to the period of time that the noise is presented. Although Chan et al. (2010b) found a long duration white noise to impair detection of a simultaneously presented visual predator, we found in Experiment 2 that 5 s after the termination of a particularly long and intense noise, the crabs were not impaired in their ability to respond to a visual predator. Rather, the acoustic stimulus *facilitated* the response to the visual predator shortly after its termination. The effects of these two processes are clearly dissociable, but presumably interact to produce relevant behavioral effects in crabs and, likely, other organisms.

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