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Decreased response or alternative defensive strategies in escape: two different types of long-term memory in the crab *Chasmagnathus*

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Abstract An opaque screen moving overhead elicits an escape response in the crab *Chasmagnathus* that after a few presentations habituates for a long period (long-term habituation). Two types of long-term habituation were previously described: the (context-signal)-long-term habituation yielded by spaced training – context dependent, cycloheximide sensitive and long lasting; and the (signal)-long-term habituation yielded by massed training – context independent, cycloheximide insensitive and shorter lasting. Present research is focused on the defensive strategies crabs display during acquisition of both long-term habituations, using video analysis as the main method of study. Aside from the escape response, *Chasmagnathus* shows a rigid motionless display, an alternative defensive response we term freezing response. The escape response is predominantly exhibited at night and in summer months, while freezing occurs during day light hours and in winter months. During acquisition of (signal)-long-term habituation, the escape response vanishes without being replaced by freezing. During acquisition of (context-signal)-long-term habituation, the escape response vanishes and is replaced by a strong freezing that finally becomes the only defensive strategy. The former, but not the latter, meets the current concept of habituation.

Key words Memory · Massed-training · Spaced-training · Habituation · Crustacea

Abbreviations *E* escape response · *F* freezing response · *LTH* long-term habituation · *ITI* intertrial interval · *R* resting · *W* wandering

Introduction

A shadow moving overhead acts as a danger stimulus, eliciting an escape response (E) in the crab *Chasmagnathus granulatus* that habituates promptly and for a long period (e.g., Pedreira et al. 1995). Mechanistic and theoretical aspects of this robust long-term memory have been extensively explored. Thus, studies were performed on its stimulus specificity (Lozada et al. 1990), adaptive value (Tomsic et al. 1993), relation to age (Tomsic et al. 1996) and circadian cycle (Pereyra et al. 1996), dependence on de novo protein synthesis (e.g., Pedreira et al. 1996), and modulation by opioids and serotonin (Godoy and Maldonado 1995; Aggio et al. 1996).

At first, the mnemonic process under study was considered to be habituation since the response decrement fulfilled most of the parametrical conditions of such nonassociative learning (Brunner and Maldonado 1988), and was termed long-term habituation (LTH) since it lasted for at least 5 days (Pedreira et al. 1995). However, further results showed that two different types of LTH can be elicited by the iterative presentation of the same habituating stimulus, depending on the number of stimulation trials and mainly on the interval between them. When a crab is given spaced training (i.e., 30 trials separated by 171 s of intertrial interval, ITI), LTH is mediated by an association between the environmental features of the training place (the context) and the features of the screen moving overhead (the signal), thus being called the (context-signal)-LTH (Tomsic et al. 1993, 1998). In contrast, when a crab is given massed training (i.e., 300 trials without ITI), LTH is not yielded by an associative learning between context and signal, depending only on the signal invariance, thus being called (signal)-LTH (Pedreira et al. 1998). Insofar as the

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research on these two instances of crab's LTH improves, their nature becomes more clearly different, both from behavioral and mechanistic viewpoints: the (context-signal)-LTH is expressed by a reduction in the level of escape response at every trial of a six-trial testing session performed 24 h after training, while the (signal)-LTH is only expressed during the last five trials of the testing session (i.e., the retraining phase of testing; Pedreira et al. 1998). The (context-signal)-LTH is cycloheximide sensitive and long lasting, while the (signal)-LTH is insensitive to cycloheximide and shorter lasting (Hermitte et al. 1999). Moreover, other mechanistic features seem to be restricted to one type of LTH; actually, (context-signal)-LTH, but not (signal)-LTH, is correlated with PKA activity (Romano et al. 1996) and κ-B-like DNA binding activity (Freudenthal et al. 1998), positively modulated by angiotensins (Delorenzi et al. 1996), and selectively regulated by a muscarinic-cholinergic mechanism (Berón de Astrada et al. 1999). Contrasting, both types of LTH are impaired when the glycosilation of proteins is blocked immediately before training (M. Saraco, personal communication).

Thus, two mechanisms different in nature would be subserving the same single process featured by a simple behavioral change, i.e., a decrement in the crab's responding that yields a long-lasting habituated response. Habituation is thought of as the deletion from the behavioral repertoire of those reactions that are not needed by the animal (Thorpe 1963). Therefore, the possibility of building up a different response as a result of the iterated stimulation is not contemplated by the current theory of habituation. According to this view, both types of crab LTH would only involve dropping of the escape response without showing other defensive response instead. Nevertheless, no systematic studies allow us to exclude such possibility with any certainty. Actually, all the research concerning crab LTH focused on the E only, considering that running away is the single defensive response of *Chasmagnathus* during the iterative presentation of the habituating stimulus. However, current observations in our laboratory suggest that not to flee from the stimulus is not necessarily tantamount to ignoring the stimulus since a motionless posture of the animal elicited by the passing screen is often detected.

Therefore, the purpose of this paper is to investigate in more detail the different items of overt behavior that the crab may display when confronted by a visual danger stimulus, and determine any correlation with each type of LTH.

Materials and methods

Animals

Animals were adults male *Chasmagnathus* measuring 2.6–2.9 cm across the carapace, weighing ca. 17 g, and collected from water less than 1 m deep in the rias (narrow coastal inlets) of San Clemente del Tuyú, Argentina. Crabs were transported to the laboratory, where they were lodged in plastic tanks (35 × 48 × 27 cm) filled to 2 cm depth with diluted artificial seawater, at a density of 20 crabs per tank. Water used in tanks and other containers during experiments was prepared using hw-Marinex (Winex-Germany; salinity 10–14 mg ml^{-1}, pH 7.4–7.6). The holding room was maintained on a 12-h light-dark cycle (lights on 0700–1900 hours). Animals were fed rabbit pellets (Nutrientes, Argentina) every 3 days and after feeding the water was changed. Temperature of both holding and experimental rooms was maintained within a range of 22–24 °C. Experiments were carried out within 15 days after the animal's arrival throughout the year. Each crab was used only in one experiment.

Apparatus

The experimental unit was the actometer (Fig. 1a): a bowl-shaped plastic container with a steep concave wall and a circular central flat floor 10 cm in diameter, covered to a depth of 0.5 cm with artificial seawater. The crab was lodged in the container which was suspended by three strings from an upper wooden framework (23 × 23 × 30 cm) and illuminated by a 10-W lamp placed 30 cm above the animal. A motor moved an opaque rectangular screen (a strip of 25 × 7.5 cm) horizontally over the animal and across the upper surface of the framework, cyclically from 1 to 2 and vice versa (Fig. 1b). A cycle of movement lasted nearly 4 s. A stylus was centrally cemented to the bottom of the container and inserted into the plastic ring of a piezoelectric transducer. Screen displacements provoked a crab's running response and as a result, container vibrations that induced electrical signals through the transducer proportional to the velocity of the vibrations. Such signals were amplified, integrated during the recording time (9 s) and translated into numerical units ranging from 0 to 1530, before being processed by computer. Thus, the scores were proportionally correlated to the velocity and number of oscillations recorded during 9 s. The experimental room had 40 actometers, isolated from each other by partitions. To avoid unobserved malfunctioning, the actometers were periodically calibrated against one another by throwing small lead balls from the upper border of the framework to the center of the container and recording the score for 9 s. A computer was employed to program trial sequences, trial duration and intertrial intervals, as well as to monitor experimental events.

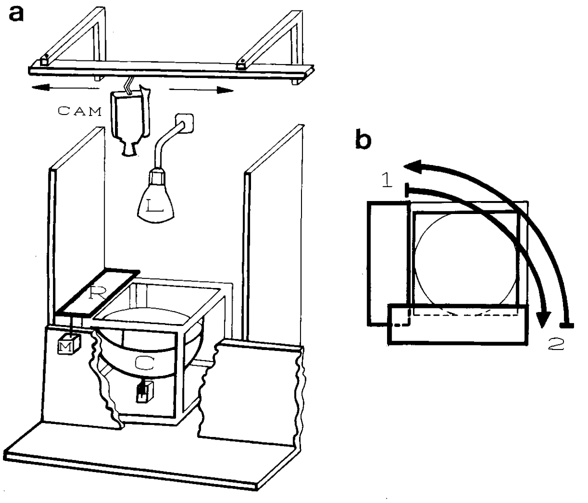


Fig. 1 a The actometer, one of the 40 units of the apparatus. *C* plastic container; *R* rectangular screen; *M* motor; *L* 10-W lamp; *CAM* video camera. **b** Movement of the screen during a cycle (from 1 to 2 and vice versa) of a trial. A cycle lasted ca. 4 s; a trial (2 cycles) ca. 9 s. The passing screen overhead is referred as *danger stimulus* or *signal*

For the video records, pictures were taken by a camera (Sony Camcorder 565) placed 40 cm above the container. The camera could be slid through the holder from one to other unit.

Experimental procedure and design

Each crab was moved from the holding room to one actometer in the experimental room. A trial consisted of two successive cycles of screen movement without rest interval between cycles, so that four passing phases per trial may be discriminated (Fig. 1b). Since each cycle lasted nearly 4 s, the total trial time was ca. 9 s. Two types of experiments are distinguished; namely, those in which the performance of the crab in the container was recorded by the actometer, i.e., the strength of the escape response was quantified according to the oscillations of the container during the entire trial time; and those in which the performance in the container was only recorded by the video camera.

In experiments involving multiple training episodes, animals were first adapted to the apparatus before undergoing training. The particular design of each type of training is described in detail along with results.

Before animals were placed in the containers to start an experiment, they underwent a selection test: each crab was turned on its back and only animals that immediately returned to their normal position were used. The rationale behind this selection is that crabs with a slow righting reaction show a low responsiveness to a large diversity of stimuli and, at a later time, they usually present unhealthy symptoms. No more than 10% of tested crabs were eliminated.

Statistics

To determine the significance of the difference between numbers of animals displaying either one or the other of two possible types of response, the binominal test was employed. When the data of an experiment consisted of percentages corresponding to two groups of animals distributed in two discrete categories, a 2 × 2 chi-square was used, but when samples were small in size the Fisher exact probability test was adopted.

Definitions

Short-term habituation refers to the response decrement within training session; *long-term habituation* (LTH) to a retention of the response decrement demonstrated in the testing session (at least 24 h after training). *Intertrial interval* (ITI) refers to the rest interval between trials. *Strong spaced training* designates an experimental protocol that includes 30 training trials with 171-s ITI and that yields LTH expressed at both the first testing trial and retraining, i.e., (context-signal)-LTH; *weak massed training*, one that includes 30 trials separated by an ITI ≤ 9 s and fails to produce LTH at any of both phases of testing; *strong massed training*, one that includes at least 120 trials, ITI = 9 s, or 300 trials without ITI, both yielding LTH expressed at the retraining phase of testing only, i.e., (signal)-LTH (Pedreira et al. 1998). The screen passing overhead is referred to as a *danger stimulus* or *signal.*

Results

Analysis of a crab's behavior before, during and after a single presentation of the danger stimulus (a single trial session)

Observations included in this section are intended to identify the different types of behaviors shown by the crab *Chasmagnathus* while remaining in the container of the actometer. For this purpose animals were studied during a 10-min pre-trial period, a 9-s trial of stimulation with the passing screen and a 10-min post-trial period.

The screen passing overhead is immediately recognized by the crab as an impending threat, eliciting a defensive response. A first main distinction may be made between the defensive responses shown during the trial and those behaviors that occur spontaneously during pre- and post-trial periods, i.e., behaviors that are not direct responses to the danger stimulus. In turn, two types of defensive strategies are distinguished: E and freezing (F); and two types of no-respondent behaviors: wandering and resting.

Defensive responses

An E consists of the crab starting to run in an attempt to move away from the passing screen, but due to the steep concavity of the circular wall, each running effort results in the crab being confined to the flat center of the container. Two main features of this response are distinguished. First, E usually starts immediately after the passing screen enters the visual field, generally preceded by a sudden short jump, and always has priority over ongoing exploratory or resting behaviors (Korn and Faber 1996). Second, an E is a directional response – specifically the crab tends to run in the opposite direction to the movement of the screen (Fig. 2).

The freezing response (F) is a rigid motionless display in which the animal lies flatted on the substratum, the second and third walking legs being held laterally extended and the chelae flexed in front of the oral area (Fig. 3b). This pattern is reached, starting from the

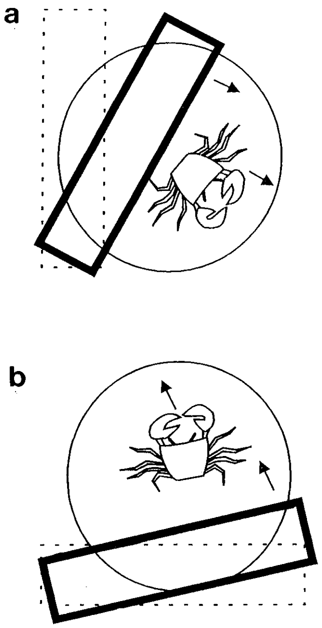


Fig. 2a,b The escape response. The crab tends to run in the opposite direction to that in which the screen is moving. **a** and **b** First and second passing of a cycle

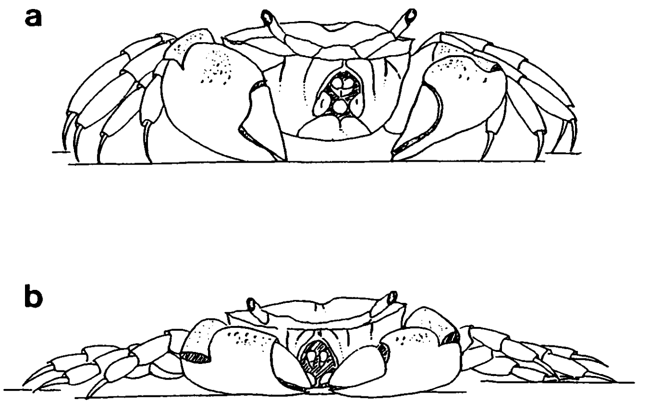


Fig. 3a,b Resting and freezing. A crab was initially in a resting position (**a**). When the screen passes overhead, a "flattening" movement starts that culminates in freezing (**b**). Drawings are based on photographs. To take the photographs, the plastic container of the actometer (*C*, Fig. 1) was replaced by a transparent one, and a camera was laterally focused on the crab

resting posture (Fig. 3a), through three simultaneous movements, i.e., first, movement of the flexed chelae until it touches the dactylus; second, lowering of the body to the substratum; and third, stretching of the second and third walking legs. These ambulatory legs are sometimes so extended and stiffly stretched that they do not touch the floor, the animal falling forward towards the anterior border of the carapace (a display we call extreme freezing). A F may be triggered, like the escape response, immediately after presentation of the passing screen, thus showing priority over ongoing no-respondent behaviors; or it may be displayed following a previous E. Exceptionally, instances of E following F were observed.

Thus, *Chasmagnathus* reacts to the visual danger stimulus in the actometer container by displaying one of two possible defensive strategies: either running away from the threat (E) or exhibiting a motionless pattern (F). A similar pair of antithetical defensive strategies is found in the behavior of very different species, including arthropods other than crabs. For instance, two predator-avoidance responses are shown in some instars of the mantid *Stagmatoptera biocellata* when confronted with a visual danger stimulus, namely, freezing or a frightening response termed deimatic reaction (Balderrama and Maldonado 1973).

To the best of our knowledge, this is the first description of a freezing response in a crustacean, though it is unlikely that this type of defensive behavior is not displayed by other crabs. In contrast, under no circumstances do *Chasmagnathus* feign death, a cryptic pattern characteristic of other crabs such as the closely associated *Helice nudus* (Hiatt 1948).

No-respondent behaviors

When *Chasmagnathus* is not confronted with the danger stimulus, one of two types of behaviors may be observed: wandering (W) or resting (R). W consists of the crab moving all around the actometer container performing an uninterrupted series of slow displacements and frequent turnings. However, very often the crab chooses to walk during W on the border between the flat center of the container and the circular wall, showing at times unfruitful attempts to climb the wall. The other type of no-respondent behavior is R, consisting of a motionless display in which the body is held raised, the walking legs flexed with the dactylus on the substratum, while the chelae are held distant each other with the propodus against the floor (Fig. 3a). R is often shown for a long while, e.g., 10–15 min, but it may be a brief intervening phase between the other two types of behavior in the container, e.g., between E and F or between W and F.

The type of defensive response at a single trial is season and time of day dependent

A single-trial experiment was performed (n = 40) during 1 day of each month (February–November) recording the number of E and F crabs. The type of defensive response the crab displayed for a longer period of time during the single trial was considered the response of the crab. The relation between the number of animals that exhibit E during a single trial and those that display F changes during the year (Fig. 4a); reaching a peak during summer and its lowest value during winter. These video analyses were performed during daylight hours (1000–1400 hours).

In order to explore the preference of crabs for E or F during darkness or daylight (2200–2400 hours and 1000–1400 hours, respectively), two single-trial experiments were conducted, one in January (summer) and the other in June (winter), with 80 animals each. The relation between E and F appears to be influenced by the time of day (Fig. 4b), i.e., there seems to be a higher preference for E at night. A chi^{2} test on these data showed a significant difference between night and day, both in summer and winter (chi^{2} = 4.1 and 3.9 for winter and summer, respectively; $P < 0.04$). For animals to be tested at night, the light of the holding room was kept on until 2200 hours.

Analysis of crab behavior during iterated presentation of the danger stimulus (multiple trial session)

The above analysis of crab behavior during a single trial indicated that the repertoire of defensive reactions elicited by the visual danger stimulus is not only constituted by the escape response. The purpose of the present analysis is to extend the study to a series of trials, in order to elucidate several questions. First, is the escape response level kept constant during repeated presentations of the stimulus? According to results from our laboratory (see review by Maldonado et al. 1997), E

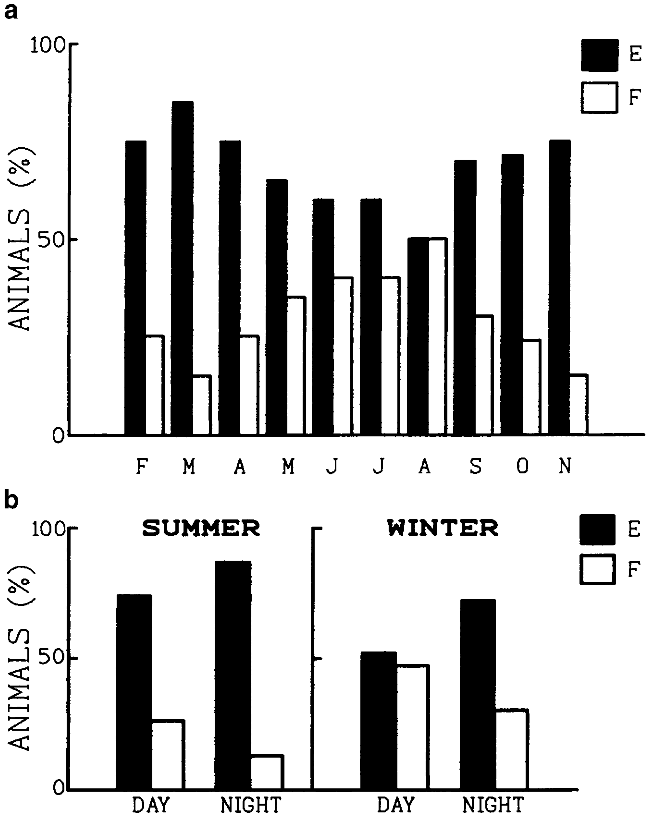


Fig. 4a,b Single-trial analysis. **a** Seasonal variation of the defensive response. *Ordinate*: percentage of crabs showing a type of response during the single-trial experiment of each month. *White bars* indicate freezing response (*F*); *black bars* indicate escape response (*E*). Forty animals per single-trial experiment. *Abscissa*: month of the year. **b** Daily variation of the defensive response. *Abscissa*: time of day. *Ordinate and other symbols* as in **a**. Eighty animals per single-trial experiment

strength, quantified by the oscillations of the actometer container, wanes during a session of habituation training; therefore, it should be expected that the probability and duration of the E, estimated by video analysis, would also decrease during the multitrial session. Second, if this is true, is the decrease in E replaced by an increase of F, or do W and R supersede both defensive responses during the trials in spite of the fact that the screen is passing overhead? Finally, does the relation between E and F during a spaced training differ from that exhibited during a massive training?

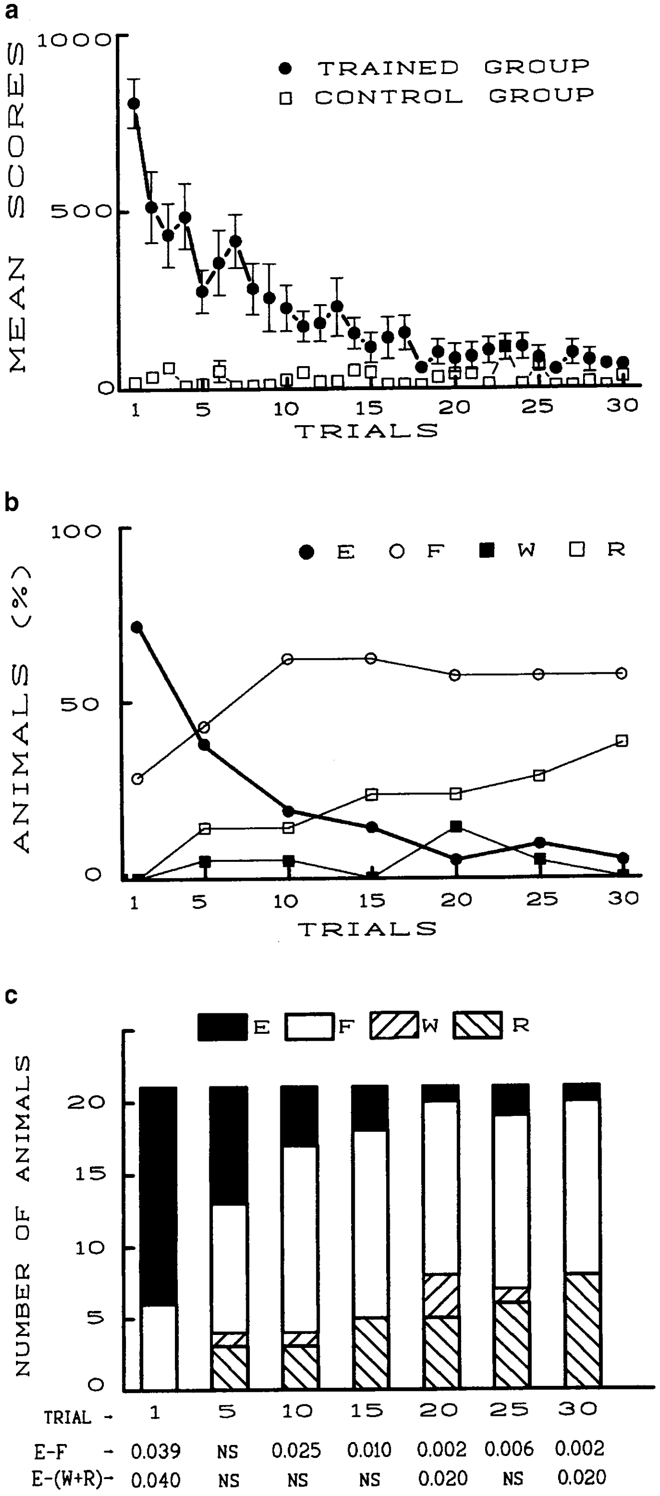
All the experiments discussed here were performed in the period November–March from 1000–1700 hours.

Changes in defensive responses during a strong spaced training

Two experiments which included a trained and a control group were conducted. In Experiment 1 (20 crabs per group), the trained group was given a spaced training protocol (30 trials separated by 171 s; preceded by 10 min of adaptation time), i.e., a training that invariably yields a robust (context-signal)-LTH at 24 h (e.g., Hermitte et al. 1999; Pedreira et al. 1998). Crabs of the control group stayed in the containers during the entire session (1 h 40 min) but without being trained. The E performances were quantified by the actometer which recorded simultaneously the activity of both trained and control group during the 9 s of each trial. During ITIs activity was not recorded. In Experiment 2 (21 crabs per group), animals from the trained group were given the above protocol but their performances were analyzed by video during trial 1, 5, 10, 15, 20, 25 and 30, and during 10 min after the last training trial. Animals were individually and successively videotaped, and the performance of a trained and a control crab was alternatively registered each time. The type of response the crab displayed for a longer period of time during each trial was considered the response of the crab for such trial. The video record of a trial lasted 9 s. Crabs of the control group stayed in the container during the entire training session, i.e., 1 h 50 min, but without being trained.

Results are shown in Fig. 5. The E strength, corresponding to the trained group and quantified by the actometer (Exp 1), waned over trials, showing the trial-response curve usually found for the strong spaced training (Fig. 5a). Negligible activity was recorded by the actometer for the control group. On the other hand, video analyses of performances corresponding to the other trained group (Exp 2, Fig. 5b) revealed a consistent reduction over trials in the number of E crabs and simultaneously, a consistent increase in the number of crabs showing F. A small number of animals displayed no-respondent behaviors (mainly R) during trial 5 and such number slightly increased during the subsequent trials. Statistical comparisons (Fig. 5c, binomial tests) disclosed a significant larger number of E than F crabs for the first trial but the number of F crabs became significantly higher than that of E crabs from trial 10 onwards. The number of animals showing no-respondent behaviors was higher than that of E crabs during trial 20 and 30. Besides, two qualitative changes in F become apparent during the strong spaced protocol. First, the stretched ambulatory legs were progressively raised to the point that during final trials some crabs fell forward towards the anterior border of the carapace, i.e., the so-called extreme freezing; and second, F persisted beyond each trial for increasing longer periods, so that an appreciable percentage of crabs remained in this display after trial 30 for a long period of time (some of them for more than 10 min). The control group of Experiment 2 showed no defensive response during all the time animals stayed in the containers, exhibiting successive periods of W and R of different time length (data not shown).

Therefore, the fall in E strength detected by the actometer during the strong spaced training is not accounted for by the progressive disappearing of the defensive responses, but mainly by the progressive building up of a strong and long-lasting F that finally becomes the only defensive strategy.



◀ **Fig. 5a–c** Multiple trial analysis. Evolution of the defensive responses during a strong spaced training (30 training trials separated by 171 s). **a** Strength of the escape responses recorded by the actometers. Control group stayed in the actometers during the entire session but without being trained. *Ordinate*: mean scores; *abscissa*: trial number. **b** Defensive response and non-respondent behaviors analyzed by video imaging. *Ordinate*: percentage of crabs showing a type of response. *E* escaping; *F* freezing; *W* wandering; *R* resting. *Abscissa*: trial number. **c** Stacked histograms based on data as in **b**. *Ordinate*: number of crabs showing a type of behavior. *Letters* for types of behavior as in **b**. *Abscissa*: first line: trial number; second line: statistical estimation of *E-F* difference per trial; third line: statistical estimation of difference *E*-(*W*+*R*)

Changes in defensive responses during a weak massed training

In the following two experiments (Exp 3 and 4) only trained groups were included, similar in all respects to the previous ones except that a weak massed training was given (30 trials separated by 9 s; preceded by 10 min of adaptation time), i.e., a training that fails to yield LTH at 24 h (e.g., Pedreira et al. 1998).

When E was estimated with the actometer, the trial-response curves obtained during the weak massed training (Exp 3, Fig. 6a), were similar to those obtained during the strong spaced training (Exp 1, Fig. 5a), although the group trained with 9 s of ITI showed a steeper slope, mainly after trial 5, a result consistent with previous reports (Lozada 1993). In contrast, conclusions drawn from the video analysis (Exp 4) were clearly different from those obtained after analyzing the strong spaced training (Exp 2). In fact, during the weak massed training E% and F% showed a drastic fall so that no defensive response was found during trial 30 (Fig. 6b). Neither E nor F extended beyond the end of a trial in any case and no instance of extreme F was presented. On the contrary, the number of crabs showing the no-respondent behaviors W and R increased steadily over trials (Fig. 6b). Statistical comparisons (Fig. 6c, binomial tests) disclosed significant difference between E and F only in trial 10, indeed both defensive responses tended to vanish; in contrast, an increasing number of animals exhibited no-respondent behaviors so that the number of W + R crabs was statistically higher than that of E crabs from trial 5 onwards. It is illustrative to contrast both statistical analyses (Figs. 5c, 6c).

Therefore, the drop in E strength detected by the actometer during the weak spaced training correlates well with the progressive disappearance of all defensive response and inversely, with the progressive rise of no-respondent behaviors that become finally the only behaviors exhibited during the presentation of the passing screen. Based on these results, it might be argued that the failure of the weak massed training to produce LTH (Pedreira et al. 1998) would be explained by the absence of a process at training that led to the build up of a consistent F as a defensive response. In other words, the development of a freezing response during training would be a requirement to acquire a robust LTH. Should this interpretation be correct, building up of F would be expected to be found in a strong massed training since it yields a robust LTH (Pedreira et al. 1998). Experiments discussed below are aimed at examining this issue.

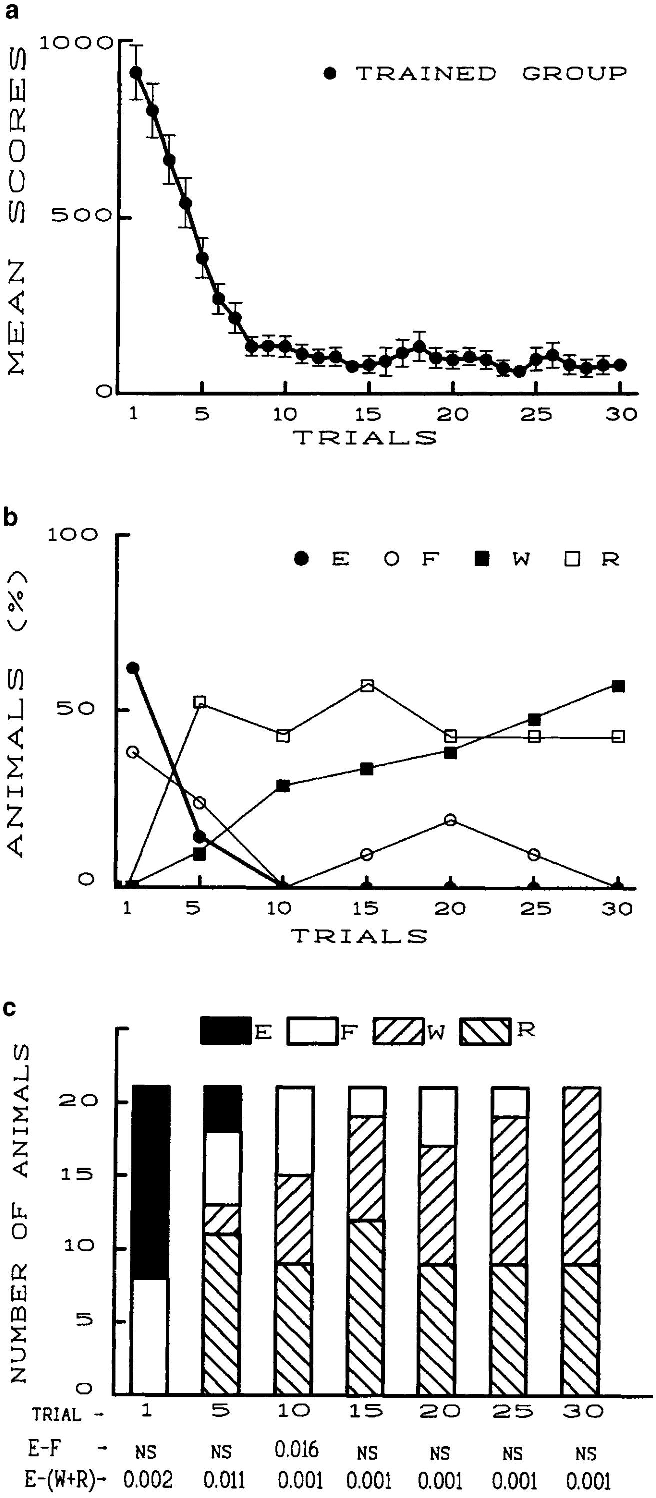


Fig. 6a–c Multiple trial analysis. Evolution of the defensive responses during a weak massed training (30 training trials separated by 9 s). **a** Strength of the escape responses recorded by the actometers. **b** Defensive response and non-respondent behaviors analyzed by video imaging. **c** Stacked histograms based on data as in **b**. *Abscissa*: first line: trial number; second line: statistical estimation of *E-F* difference per trial; third line: statistical estimation of difference *E*-(*W*+*R*). Symbols as in Fig. 5

Changes in defensive responses during a strong massed training

A massed training, i.e., a training with trials separated by an ITI of <9 s, induces (signal)-LTH at 24 h provided that a large number of trials are given, e.g., 120 trials with ITI = 9 s or 300 trials with ITI = 0 s (Pedreira et al. 1998; Hermitte et al. 1999). This type of training is called strong massed training and it is used to test if F is built up during the repeated presentation of the danger stimulus.

The experiment (Exp 5) included two groups of crabs ($n=20$). One group (the spaced group) underwent 30 training trials separated by 171 s of rest interval while the other group (the massed group) was given 300 trials without ITI. Video analysis was made on performances during trial 15 and 30 of the spaced group and on those during trial 300 in the massed group.

When the analysis was focused on the last trial (Fig. 7a), i.e., on trial 30 of the spaced group and on trial 300 of the massed group, few crabs with E were found in both groups, but while most of the responses were F after spaced training, most of the cases were of no-respondent behaviors after massed training. A Fisher exact probability test performed on these data confirmed this observation, since it showed that animals of the spaced group and those of the massed group did differ in their preference for either the defensive response F or the no-respondent behavior (W + R) ($P<0.001$). In other words, the fall in the number of E crabs in the spaced group is explained by a sharp increase in the number of crabs with F; while the fall in the massed group is explained by a sharp increase in the number of crabs showing no-respondent behaviors. However, it might be argued that such differences between groups would not be due to the different type of training protocol but to the fact that the stimulation phase is longer for the spaced training (1 h 30 min) than for the massed training (45 min). For the purpose of testing this explanation, the analysis was focused on results obtained after a stimulation phase of the same length (45 min), i.e., in trial 15 for the spaced group and in trial 300 for the massed group (Fig. 7b). A result similar to that shown in Fig. 7a was obtained, disproving the alternative explanation. In addition, unlike the strong spaced training, no instances of extreme F were found during the strong massed training.

Therefore, during a session of 300 trials without ITI no building up of F seems to take place, though this massed training invariably yields (signal)-LTH at 24 h (Pedreira et al. 1998). In other words, the construction of a F is required during the acquisition of (context-signal)-LTH, but not during the acquisition of (signal)-LTH.

Discussion

This study is discussed firstly with respect to the results obtained from analysis of the crab's behavior in a single

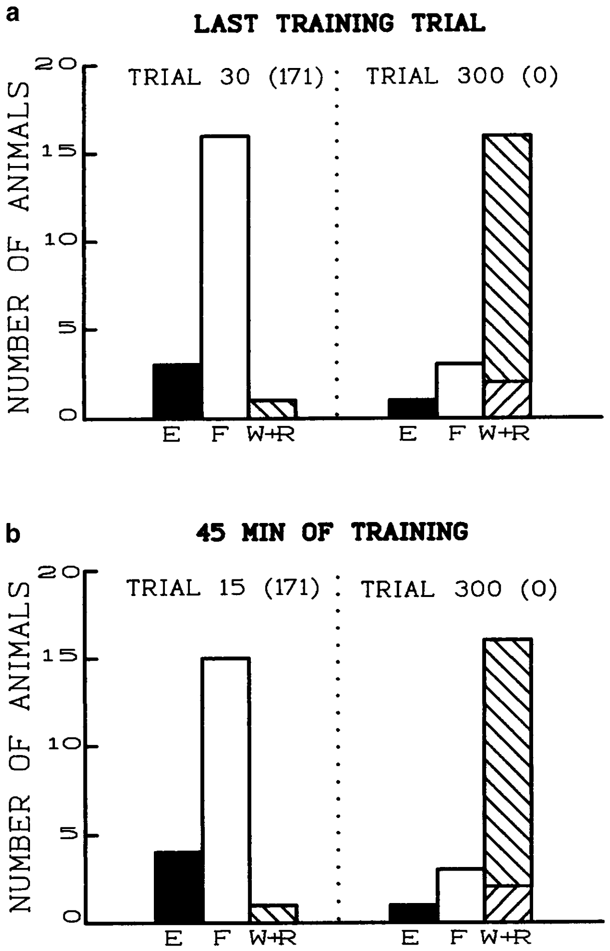


Fig. 7a, b Multiple trial analysis. Comparison of strong spaced versus strong massed training (30 training trials separated by 171 s versus 300 training trials without intertrial interval). **a** Performances during the last trial of each training. *Left panel*: trial 30 of the strong spaced training, i.e., after 1 h 30 min of training; *right panel*: trial 300 of the strong massed training, i.e., after 45 min of training. **b** Performances after equal training time (45 min) for each group. *Left panel*: at trial 15 of the strong spaced training; *right panel*: at trial 300 of the strong massed training. Symbols as in Fig. 5

trial and secondly, with respect to results of analysis of a session in which the danger stimulus is repeatedly presented.

Video analysis of the crab's performance before, during and after a single trial, lead us to conclude that the repertoire of defensive reactions elicited by the visual danger stimulus is not only constituted by the escape response. A rigid motionless display we termed F proves to be the alternative strategy of the crab when it is confronted with a visual danger stimulus. The preference for one of the two strategies shows seasonal variation. E is predominantly chosen during summer months, but this gives way to F and reaches a minimum during winter months. A parallel variation in some physiological and behavioral parameters of *Chasmagnathus* may be mentioned. During winter the metabolic rate estimated by oxygen consumption reaches a minimum (Rosas et al. 1992; D'Incao et al. 1988a,b); muscle lipids instead of hepatopancreas or muscle glycogen become the main energy substrate (Kurcharsky and Da Silva 1991); and crabs tend to remain for longer periods in their burrows (D'Incao et al. 1988b). Coincidently, the frequency of diet items in the stomach of the animals is clearly reduced (D'Incao et al. 1988a), i.e., all changes apparently save energy in winter months. However, to conclude that a relative increase of the F is in keeping with the assumed adaptive value of other physiological changes, it remains to be demonstrated that F entails a lower use of energy.

Aside from the seasonal variation in preference for the defensive strategy, there seems to be a daily pattern of variation, namely a stronger tendency to escape at night regardless of the season. This result is consistent with previous studies reporting that either reactivity of *Chasmagnathus* to a danger stimulus or locomotor activity, both recorded during the night phase, exceed that observed during the light phase (Pereyra et al. 1996). Parallel day-night variations in three main physiological parameters have been reported in brachyuran crabs, namely blood glucose level (e.g., Dean and Vernberg 1965; Rosas et al. 1992), oxygen consumption (Rosas et al. 1992) and increase in the release of the crustacean hyperglycemic hormone (e.g., Kallen et al. 1990). Therefore, the peaks of preferences for escaping as defensive response seem to agree with those periods in which a generally higher level of metabolic rate is shown, i.e., summer months and hours of darkness.

When the crab's behavior is observed throughout several trials, a graded decrease in response to the repeated danger stimulus results, regardless of the extension of the ITI. In other words, there is a clear-cut, short-term habituation during either spaced or massed training. The only difference between both types of training becomes apparent in the temporal course of the short-term habituation; specifically, the wane in responding is steeper for massed than for spaced training (Romano et al. 1991). However, the video analysis in the present study disclosed a conspicuous difference between both processes. While during the massed training the E vanishes without being replaced by the other defensive response, during the spaced training it is substituted by an increasingly stronger and longer F display. The former fits well with the current definitions of habituation, either "learning what not to do" (Razran 1971), "cessation of responses" (Treinsman 1984), or "adaptation to the decreasing significance of a stimulus" (Braun and Bicker 1992). Conversely, the latter entails the building up of an alternative defensive response instead of fleeing from the danger stimulus; namely, a change of strategy within the repertoire of defensive responses.

Thus, a further and conclusive difference between LTH acquired by massed training and that acquired by spaced training is apparent: the (context-signal)-LTH entails the building up of a strong and persistent F during acquisition, while the (signal)-LTH, involves a simple vanishing of the E. The latter meets the current concept of habituation but the former may hardly be considered as an instance of habituation. Instead, the (context-signal)-LTH appears more adequately defined as an associative memory process; specifically, a process mediated by an association between contextual cues and the eliciting stimulus (Tomsic et al. 1998). However, it is pertinent to consider an alternative explanation of the results in terms of an associative/operant conditioning. It might be argued that vibrations of the chamber, though small since the chamber is restrained by the insertion of the stylus into the piezoelectric, produce aversive reinforcement which could be associated with the E. Given that F avoids the reinforcement, crabs would tend to replace escape by freezing. Recent results from our laboratory rule out this interpretation. A new experimental set up, identical in all respects to that used in this paper but with motionless chambers, was specially designed for video analysis of the crabs' response. In a series of experiments aimed at a further characterization of the LTH processes, the new equipment was used, and results obtained during training sessions were entirely in keeping with those of the present paper.

The foregoing set of findings may be taken as a rather extreme instance of an apparently universal phenomenon in memory formation, namely the ITI effect (e.g., Hawkins et al. 1993). The interaction between trial spacing and the strength of the memory retention has recently received a great deal of theoretical attention (e.g., Schmidt and Bjork 1992; Rogers et al. 1994) and different explanations have been proposed. No mechanistic explanation of the ITI effect in the formation of the two different types of long-term memory in *Chasmagnathus* may be yet offered, but it is tempting to interpret the difference between spaced and massed training in adaptive terms. In other words, it might be assumed that the repeated presentation of a passing screen, separated by rest intervals, may be likened to a predator passing overhead, while the same stimulation without intervals would hardly be representative of a predator. More likely, the uninterrupted presentation might mimic the continuous oscillations of the dense vegetation of cord grasses that often constitutes the crab's habitat (Tomsic et al. 1993). If so, clearly different lines of behavior are expected to be adopted for each case. The continual presence of a predator (i.e., spaced training) results in a behavior that although ensuring the continuity of the defensive strategy, replaces the conspicuous E by cryptic F. Moreover, a memory of the context in which the predator appeared is acquired. In contrast, the uninterrupted stimulation (i.e., massed training), like that produced in the wild by the oscillations of cord grasses due to a storm, would result in a lessening of the initial escape response without it being replaced by other defensive strategies. In addition, a shorter-lasting retention of the habituated response is acquired. The (context-signal)-LTH would operate chiefly to ensure the defense of the crab by means of a more economical strategy, and the (signal)-LTH to save energy as well as time to perform maintenance activities. Thus, though both mnemonic processes are triggered by the same danger stimulus, the meaning associated with the stimulus and the type of memory that develops, would depend on how the stimulus is presented during the trials.

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