Pharmacology Biochemistry & Behavior, Vol. 36, pp. 787–793. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Central Effect of Morphine Pretreatment on Short- and Long-Term Habituation to a Danger Stimulus in the Crab *Chasmagnathus*

DANIEL TOMSIC AND HECTOR MALDONADO[1]

Laboratorio de Fisiologia del Comportamiento Animal, Facultad de Ciencias Exactas y Naturales UBA, Avda Indalecio Chenaut 1910 (1426), Buenos Aires, Argentina

Received 22 August 1989

TOMSIC, D. AND H. MALDONADO. *Central effect of morphine pretreatment on short- and long-term habituation to a danger stimulus in the crab* Chasmagnathus. PHARMACOL BIOCHEM BEHAV **36**(4) 787–793, 1990.—Morphine is believed to inhibit the crab's escape response to a danger stimulus due to central drug action. To test alternative explanations of such an effect in terms of afferent and/or efferent impairment, experiments were conducted using the crab's optokinetic response as indicator. Doses of morphine with maximal detrimental effect on the escape response (75–100 μg/g) showed no effect on the optokinetic response, both by measuring the crab's eyestalk displacement and by recording its body rotation, supporting the hypothesis of a morphine central action on the danger-induced escape response. As regards the effect on habituation, a 75 μg morphine/g injection administered 30 min before the first trial produced a parallel shift of the short-term (within-session) habituation curve, suggesting a modulatory central drug action that would mimic a putative endogenous opioid action. A 100 morphine μg/g dose injected 30 min before training sharply reduced reactivity during training and impaired the acquisition of long-term (between-session) habituation. It may be speculated that the decrease in the danger meaning of the stimulus due to morphine explains both effects in terms of a stimulation impairment during training.

Opiates Morphine Short-term habituation Long-term habituation Optokinetic response Danger stimulus Crustacea

TWO characteristic reactions of the crab *Chasmagnathus granulatus* have been widely studied in our laboratory: the *defensive reaction* and the *escape response*. The defensive reaction is a display in which both chelae are spread to the sides of the carapace, held in an extended position, and the carapace elevated on the flexed walking legs [lateral merus display: (20,23)]. This response is a well-known reaction of a crab in nature, when facing a close danger or receiving a strong aversive stimulus, and in our laboratory it was induced by an electrical shock given through implanted electrodes (10). The escape response is a sudden running usually elicited by a rather mild aversive stimulus or by a distant danger, and in our laboratory was provoked by a mild electrical shock given through the water or by a rectangular screen moved horizontally or vertically overhead. Repeated screen presentations produce an escape response decrement that meets most of the parametric criteria of habituation (2).

Opioid action on such crab responses has been increasingly reported. Morphine administered to *Chasmagnathus* 30 min before stimulation with an electrical shock produced inhibition, naloxone reversible, of the defensive reaction (10). Morphine action on the crab's escape response elicited 30-min postinjection by a moving screen, showed a dose-dependent reduction, naloxone reversible (13). Crabs injected with naloxone 15–30 min before a session of habituation to a rectangular screen stimulus showed a higher response level versus controls (17). Repeated stimulations with a horizontal rectangular screen decreased the reactivity to both an electrical shock and to a vertical rectangular screen, but either effect was abolished by naloxone pretreatment (18,21). Such findings give rise to the following considerations.

Firstly, opioid receptors appear to mediate morphine action in *Chasmagnathus* as shown by previous work concerning morphine-induced analgesia in other arthropods, using a pain-induced defensive behavior as end point [e.g., (8, 15, 24)].

Secondly, morphine seems to inhibit in this crab both a response elicited by a painful stimulus (an electrical shock) and one induced by a painless (danger) stimulus (a rectangle moved horizontally or vertically). Whereas the former case is consistent with the well-known antinociceptive effect of this drug in diverse

[1]Requests for reprints should be addressed to H. Maldonado, Laboratorio Fisiologia del Comportamiento, Avda, Chenaut 1910 (1426), Buenos Aires, Argentina.

species, the latter action is at odds with the generally accepted view that opiates have no effect on the reactivity to danger or fear stimuli [e.g., (6, 7, 22)]. Therefore, it is worthwhile to determine whether the detrimental effect of morphine on the crab's reactivity to a danger stimulus is mainly due to a depression of the afferent and/or efferent paths (peripheral drug action), or, alternatively, to a central action.

Thirdly, the crab's habituation to a danger stimulus appears to involve the action of an endogenous opioid mechanism. Should such a hypothesis prove viable, an analysis of the effect of morphine pretreatment on both the short-term (within-session) and long-term (between session) habituation, might shed light on the modalities of the putative endogenous opioid action.

Accordingly, the purpose of the present paper is two-fold. First of all, to address the key question (Experiments 1 and 2) whether the described inhibitory effect of morphine on the escape response elicited by a danger stimulus is a central effect; and if this is actually the case, to study such a central inhibitory action on short- and long-term habituation to a danger stimulus (Experiments 3 and 4).

EXPERIMENT 1

To unravel the question whether morphine effect on the reactivity to a danger stimulus is a peripheral or a central drug action, an ideal approach would be to study the effect on a crab's escape response elicited neither by a painful nor a danger stimulus. Such an ideal experiment seems difficult to design since an escape response can hardly be elicited by a neutral visual stimulus. However, a response might be employed which, without being identical to an escape response, involved noticeable motor activity without requiring either a danger or a painful stimulus. The crab's optokinetic response, in which movements of the eye stalks and the body compensate for image displacements over the eyes, fulfils these conditions.

Accordingly, Experiment 1 was designed to test the morphine effect with the 2 higher doses previously used (13), but employing as indicator the optokinetic response evoked by rotating a large contrasting visual field (9,19) and quantifying potential drug effect on the efferent and/or the afferent limb of the nystagmus.

METHOD

Animals

Animals used in the 4 experiments of the present paper were adult male *Chasmagnathus granulatus* crabs, 2.8–3.0 cm across the carapace, collected from water less than 1 m deep in the *rias* (narrow coastal inlets) of San Clemente del Tuyu, Argentina, and transported to the laboratory, where they were lodged in glass tanks (35 × 48 × 27 cm) with walls painted black and with a 1–2 cm depth of water, brought from the same place animals had been captured. Females were not used given their uneven behavior during the year, as shown by sharp decreases in reactivity during egg maturity and oviposition periods (observations in our laboratory).

Mean crab weight was determined as described elsewhere (2) (17.3 g, SE 0.2, n = 60) and absolute drug doses calculated according to this mean. Holding room and experimental room were kept at constant temperature (18–20°C). Experiments were conducted 1, 2 or 3 days after animals' arrival. Each crab was only used in one experiment.

Apparatus and Procedure

A full description of the apparatus is in the caption of Fig. 1a, b. The crab was moved from the holding room to the experimental room, where it was secured in the clamp, the flag cemented on its eye, and the needle implanted. Then, clamp plus crab was mounted on the platform (inside the drum) and there moved up to find the position where the photocell provided the resting reading.

After 15-min adaptation, the first trial (T1) was run. Each trial consisted in switching on the light and the drum motor during 2 min, recording the eye movements only for the second min. The injection was administered immediately after T1, followed by a second trial (T2) after an interval of 30 min. The choice of this interval was based on previous work with painful and danger stimuli (10,13).

Response level was measured as response frequency RF (number of amplitude peaks per trial) and response amplitude RA (average of the peak amplitudes per trial). For data analysis, relative percentage of responses during the first and second trial were considered, i.e., RF% = RF2/RF1 × 100, and RA% = RA2/RA1 × 100.

During pilot experiments, neither the response frequency nor the response amplitude were markedly different from 100% either for crabs injected with saline or for those which remained uninjected. Sixty crabs were randomly assigned to 3 groups of 20 each: the SALINE group was injected with the vehicle, the MP75 with 75 μg/g of morphine (morphine-HCl, Saporiti, Argentina), the MP100 with 100 μg/g. Rationale for the choice of these 2 morphine doses was that they were the higher doses with inhibitory effect 30 min after injection used in previous experiments (13).

RESULTS AND DISCUSSION

A one-way ANOVA performed on data from the 3 groups revealed no significant differences either between the mean RF% values ($F = 0.98$) or between the mean RA% values ($F = 0.75$). Thus, neither the crab's visual accuracy nor the motor ability of its eye stalks are significantly impaired by injecting a morphine dose equal to or lower than 100 μg/g. This finding suggests that the inhibitory effect previously demonstrated (13) on the escape response elicited by a danger stimulus is hardly attributable to visual disability. However, the lack of impairment in fine eye stalk movements fails to rule out motor disability as an alternative explanation.

EXPERIMENT 2

To further address the possibility of detrimental morphine effect on the crab's general motility, the following experiment was conducted.

METHOD

They were as described for Experiment 1, though some changes both in experimental device and procedure were introduced. A transparent cylinder (20 cm high, 15 cm in diameter), whose base had 8 evenly spaced marks 45° apart, replaced all the devices formerly located on the disc (Fig. 1b). The crab's eye stalks were fixed with a dab of epoxy cement and the animal placed in the transparent cylinder. Thus, the optokinetic response was expressed here as a rotation of the crab following the movement of the stimulus drum. After 15 min of dark adaptation in the cylinder, the first trial (T1) was carried out. A trial consisted of switching on the light and the drum motor for 2 min and recording the crab's displacement for the second min. This record was performed by simple observation, counting the number of marks on the cylinder base crossed by the left eye stalk during a trial. An injection was administered in the usual way immediately after T1, and then, after 30 min in darkness, a second trial (T2) was run. Results are expressed in relative values (percentage of

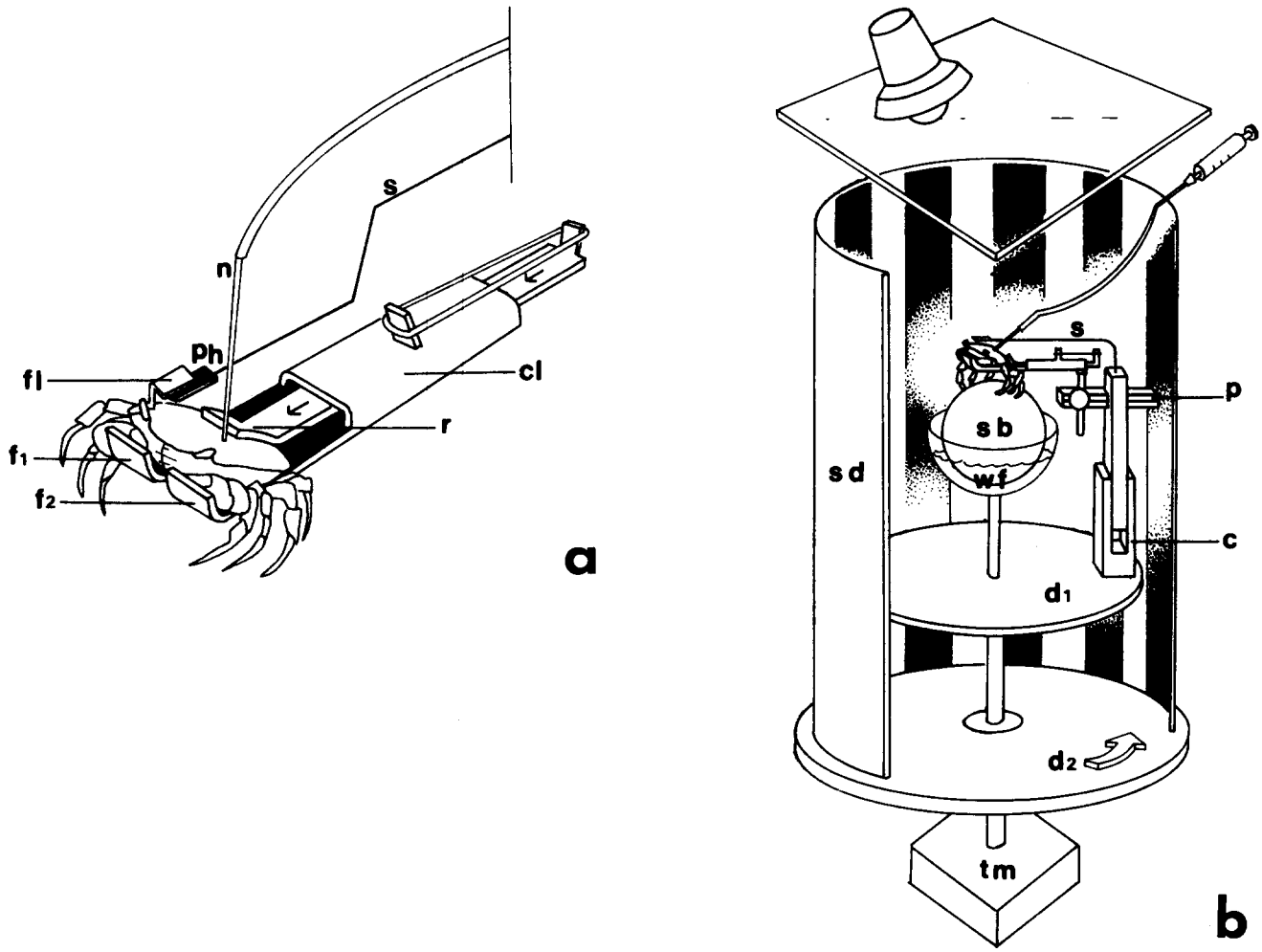

FIG. 1. (a) Experimental device to secure the crab. (b) General view of the apparatus. The crab was held in the stimulus drum by a specially devised clamp. A rear mobile flange (r) in the clamp was slid forward by a rubber ring, rigidly holding the crab against the frontal flanges (f1-f2) and preventing claws from moving. The clamp was mounted on an universal ball bearing, which could be moved to and fro on platform (p) and up down on column (c), so that the crab could be easily placed in the center of the stimulus drum (sd) and in a proper position as regards the photocell (ph). The crab's legs were supported by a styrene ball (sb), diameter 15 cm, floating in the water of a bowl (wf), which could be rotated by the crab walking on the spot. Rationale for this device was that the crab's optokinetic response proved roughly five times more powerful when walking than when resting (13). Both bowl and column were mounted on the rigid disk (d1). The stimulus drum (diameter = 28 cm, height = 38 cm) was supported by the mobile disc (d2), directly connected to the driving axle of a torque motor (tm), which rotated at 2 r.p.m. [a stimulus speed that induces strong optokinetic responses (9)]. Light from an incandescent lamp of 75 W, 10 cm above the drum, was passed through a transparent glass to reduce crab heating. The stimulus pattern consisted of 9 evenly spaced vertical black stripes, each one 20° wide. In order to record eye movements, a light aluminum flag (fl), weighing 2.5 mg, was secured to the top of the right eye by a dab of epoxy cement. The photocell, looking upward, was fixed on the wire support (s). By moving the clamp properly, the crab was placed in such a way that the flag remained closely above the photocell. Yaw displacements of the eye induced voltage changes in the photocell circuit which, after being amplified, were processed by a computer. For each experiment, a printer plotted a displacement versus time curve, indicating the highest and lowest amplitude values on a 0–250 scale. To inject morphine or the vehicle (NaCl solution, 1.6%), the crab had a stainless steel needle (n) implanted through a specific point of its carapace (2), 3 mm deep, and connected by a Teflon tube to a syringe which was clasped in an external support. The syringe was filled with the required solution (100 μl).

rotation, R% = R2/R1 × 100).

Sixty crabs were randomly assigned to 3 groups of 20 each; the SALINE group injected with the vehicle, the MP75 with 75 μg morphine/g and the MP100 with 100 μg/g.

RESULTS AND DISCUSSION

A one-way ANOVA performed on data from the 3 groups revealed no significant difference between the mean percentages of rotation for each group. Thus, doses having no significant effect on the nystagmus response measured by eye stalk displacements (75 and 100 μg/g) proved likewise ineffective when the response was measured by crab's rotation.

Results from Experiments 1 and 2 seem to rule out an explanation of the morphine action on the reactivity to a danger stimulus, in terms of a detrimental effect of morphine on visual and/or motor ability. Actually, the highest doses that affect the escape response to a rectangle moved horizontally, 30 min postinjection (13), fail to inhibit nystagmus following similar injection-test interval. Therefore, it may be concluded that the effect of morphine does depend specifically on the type of visual

stimulus presented, i.e., morphine seems to inhibit only those responses elicited by a visual stimulus meaning danger.

EXPERIMENT 3

A recent report (18) indicated that morphine injected immediately after repeated presentation of a danger stimulus fails to impair the crab's long-term habituation. No studies, however, have been conducted to investigate the pretreatment morphine effect on short-term habituation. The point has special relevance because results from Experiment 1 and Experiment 2 suggest that the inhibitory morphine action in the escape response is a central one, and in addition, because an endogenous opioid mechanism acting during short-term habituation has been proposed (17).

Thus, Experiment 3 was aimed at studying the morphine pretreatment effect on the habituation to a danger stimulus. An analysis of alterations in habituation time-course might help to understand the mechanisms that subserve response changes during stimulus repetition. Actually, Davis and File (4) have predicted that administration of a drug should produce a change in the slope of the response-trial curve provided that intrinsic habituation is altered, but only a parallel shift when the drug alters systems that have a modulatory action.

EXPERIMENT 3A

METHOD

Apparatus

The apparatus used is described in detail elsewhere (17). Briefly, the experimental unit was the *actometer*: a bowl-shaped plastic container with sharply concave walls suspended by three strings from an upper wooden framework (23 × 23 × 30 cm), illuminated by a 10-W lamp, and its bottom in contact with a phonograph needle. The crab was lodged in the container whose floor was covered to a depth of 1 cm in sea water. A motor displaced an opaque rectangular screen (25 × 13 cm), almost touching the upper border of the framework, at such an angular speed that it ran diagonally over the entire aperture in 2.3 sec, eliciting an escape response from the crab. Such a response was, however, limited to the flat center of the container, since the sharp concavity of the walls prevented the animal from climbing them. Container movements caused by the crab's reaction induced voltage changes in the piezoelectric element of the phonograph needle, which were amplified, integrated and translated into numerical units ranging from zero to 1020. Amplification was chosen in such a way that the maximum score remained below 1020 units, which were processed by a computer. The spontaneous activity of the crab was sporadic and very slow, so that scores no higher than 10 units were recorded when no screen-induced movement occurred. Scores greater than 50 units were considered indicative of an escape response. The experimental room had 40 actometers, quite isolated from each other by lateral partitions and a frontal wall.

Procedure

Each crab was moved from the holding room to the experimental room, injected by means of a Hamilton syringe through the cephalothoracic-abdominal membrane and then placed in the container of the actometer. Injections consisted of 100 μl of the vehicle (NaCl, 1.6%) or a morphine solution. After 30 min adaptation in the actometer, a 15-trial session started. Each trial consisted of passing the screen 4 times over the actometer. Trial time was 9 sec and the intertrial interval 27 sec. Crab activity was recorded during the entire trial time.

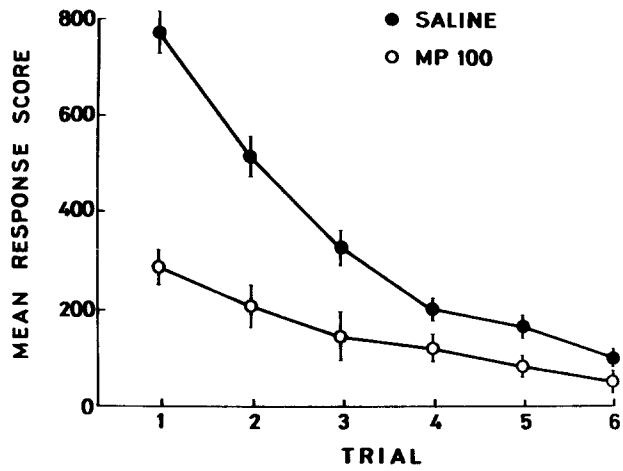

FIG. 2. Pretreatment morphine (100 μg/g) effect on short-term habituation. Six first trials of a 15-trial session. Injection–1st trial interval: 30 min.

Eighty crabs were randomly distributed in 2 groups of 40 each: the SALINE group injected with the vehicle, and the MP100 with 100 μg morphine/g solution. Both groups were run together.

RESULTS AND DISCUSSION

Figure 2 depicts the response-trial curves of both groups. The analysis is restricted to the first 6 trials, since in agreement with previous findings (11,12) there was a consistent gradual fall up to the 6–7th trial and a stable asymptotic level thereafter. An ANOVA (mixed-repeated measures, 2 × 6) performed on these data disclosed a significant main drug effect, $F(1,78) = 24.8$, $p<0.005$, a significant effect of the trials, $F(5,390) = 90.9$, $p<0.005$, and a significant drug × trial interaction, $F(5,390) = 23.4$, $p<0.005$. Regression linear analysis, calculating the equation for each subject of each group, yielded a mean slope of -133.9 (± 9.9) for the SALINE group and of -50.5 (± 8.0) for the MP100 group, providing a highly significant intergroup difference, $t(78) = 6.6$, $p<0.005$.

Thus, a dose of 100 μg morphine/g fails to produce a parallel shift of the curve but leads to a sharp reduction in slope. However, a parallel shift might be masked by the strong depressing action of the dose along with a floor effect. Therefore, a smaller dose (75 μg/g) was tested in Experiment 3B.

EXPERIMENT 3B

METHOD

Apparatus and Procedure were the same as in Experiment 3A. Eighty crabs were randomly assigned to 2 groups of 40 each: the SALINE group injected with the vehicle and the MP75 group with 75 morphine μg/g. Both groups were run together.

RESULTS AND DISCUSSION

Figure 3 shows both curves. The analysis is restricted, as in Experiment 3A, to the first 6 trials. A cursory inspection to this figure suggests that this dose produced a parallel shift. An ANOVA (mixed-repeated measures, 2 × 6) on the data confirmed such a conclusion: there was significant drug effect, $F(1,78) = 11.3$, $p<0.005$, and significant effect of the trials, $F(5,390) =$

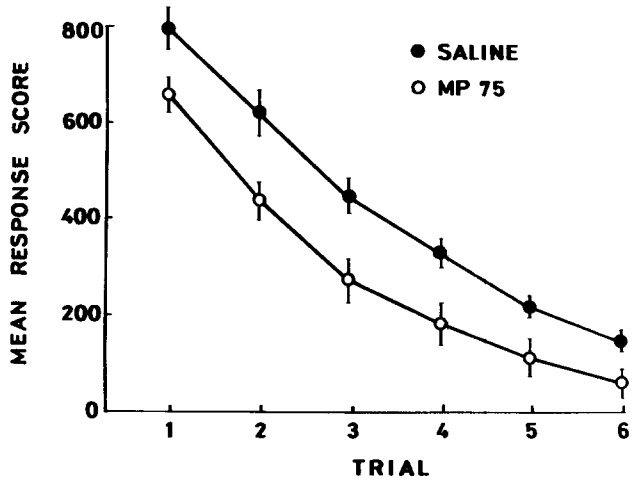

FIG. 3. Pretreatment morphine (75 μg/g) effect on short-term habituation. Six first trials of a 15-trial session. Injection–1st trial interval: 30 min.

139.5, $p<0.005$, though no significant drug × trial interaction, $F(5,390)=0.87$. Regression individual analysis performed on responses of both groups showed a mean slope of −129.5 (±9.3) for the SALINE group vs. −114.9 (±9.5) for the MP75 group, and the *t*-test indicated no significant intergroup difference, $t(78)=1.1$.

According to the Davis and File proposal (4), this parallel shift in the response-trial course, 30 min after morphine injection, is indicative of a modulatory drug action. In other words, the short-term (within-session) habituation itself would not be altered, so that the same response decrement slope should be expected regardless of drug dose. However, such a constant value cannot be demonstrated with a higher morphine dose (100 μg/g) due likely to a floor effect.

EXPERIMENT 4

Two alternative hypotheses may account for the central modulatory drug action proposed above. As previously advanced (13), the morphine effect would result from an interference with the decoding of the danger signal, namely by a reduction in the magnitude of the danger that the stimulus normally signals. On the other hand, an alternative proposal would explain the morphine effect by a raising of the response threshold to a danger stimulus.

It is widely accepted in habituation studies that response is hardly a critical factor in long-term habituation. Response-independent habituation has been reported several times (1, 3, 5, 16), and instances of subzero habituation support the proposal that acquisition could take place even though responses were suppressed or greatly inhibited during training. In agreement, Lozada *et al.* (12) have provided evidence indicating that the degree of long-term habituation to a danger stimulus (24-hr between-session interval) in *Chasmagnathus* correlates with the number of stimulations during training but neither with the response level nor with the waning response rate. Bearing this in mind, an experiment aimed at estimating the action of morphine, administered 30 min before a training session, on long-term habituation, would help to decide which of the two above explanations of morphine action has more predictive value. Actually, results showing no long-term habituation would support the hypothesis that morphine decreases the danger meaning of the stimulus, because stimulation is impaired during training. In contrast, results showing acquisition would support the hypothesis of response threshold raising, because despite a low response level during training, a meaningful stimulus is present. To that end, Experiment 4 was conducted to study the morphine effect on long-term habituation.

METHOD

Apparatus and Procedure

The same apparatus of the foregoing experiment was used. The basic procedure was also as that described for Experiment 3, though here two 15-trial sessions were given: the *training* session and the *testing* session, separated by a retention interval. A single morphine or saline injection was given 30 min before the *training* session.

Pilot experiments on a group of animals injected with saline and another with 100 morphine μg/g, and tested several hours later with a single 15-trial session, showed that only after 4 hr and 30 min the inhibitory effect of the drug had wholly disappeared. Therefore, such a period was used as retention interval.

One hundred and sixty crabs were randomly assigned in equal number to each cell of a 2×2 factorial, the factors being drug (SALINE: the vehicle, or MP-100: 100 morphine μg/g) and *training* session (0: no *training* session, or TR: *training* session), so that 4 groups were formed: SALINE-0, SALINE-TR, MP100-0, and MP100-TR. Groups were run together, i.e., 0- and TR-groups were placed in the actometers simultaneously, remaining in the apparatus during the entire retention interval.

RESULTS AND DISCUSSION

A comparison between performance of SALINE-TR and MP100-TR during the *training* session disclosed results like those obtained in Experiment 3A, i.e., there was a sharp inhibitory effect of morphine pretreatment on the training response level recorded 30 min after injection. On the other hand, the response level difference of SALINE-0 vs. MP100-0 during the *testing* session was not significant, $F(1,78)=0.45$, i.e., the inhibitory effect of morphine pretreatment had wholly disappeared during the *testing* session.

Figure 4 presents mean response scores to the screen vs. consecutive 3-trial blocks for both the SALINE-0 and SALINE-TR. Scores for each block were obtained by averaging accumulated scores per animal for each of 3 consecutive trials. An ANOVA (mixed-repeated measures, 2×5) performed on these data showed a significant effect of the training factor, $F(1,78)=17.0$, $p<0.005$, the trial blocks factor, $F(4,312)=227.4$, $p<0.005$, and the training × trial blocks interaction, $F(4,312)=11.6$, $p<0.005$.

Performances of MP100-0 and MP100-TR are illustrated in Fig. 5, where mean values were calculated as in Fig. 4. Curves proved quite similar to each other and no significant effect of either the training factor, $F(1,78)=0.96$, or the training × trial blocks interaction, $F(4,312)=1.4$, $p>0.25$, was disclosed by the ANOVA.

Thus, the 15-trial training session produces a distinct long-term habituation after an interval of 4 hr and 30 min (SALINE-TR). In contrast, exposure of animals injected with morphine 30 min before training to the same number of training stimulations (MP100-TR), fails to produce a response decrease after an identical intersession interval. This sharp difference, despite the same stimulus exposure, is consistent with the hypothesis that explains the morphine effect on the danger-induced escape response by a weakening of the stimulus-meaning link.

GENERAL DISCUSSION

A first conclusion from this paper (Experiment 1 and Experi-

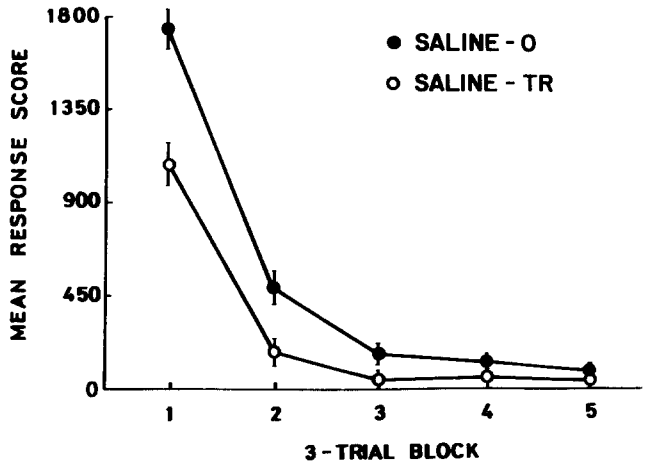

FIG. 4. Performance of saline-injected groups during a 15-trial *testing* session. Mean response score stands for the average for accumulated scores of each block of 3 trials. The SALINE-0 group had received no training after a saline injection given 30 min before. The SALINE-TR group had received a 15-trial training session 30 min after injection. Retention interval: 4 hr 30 min.

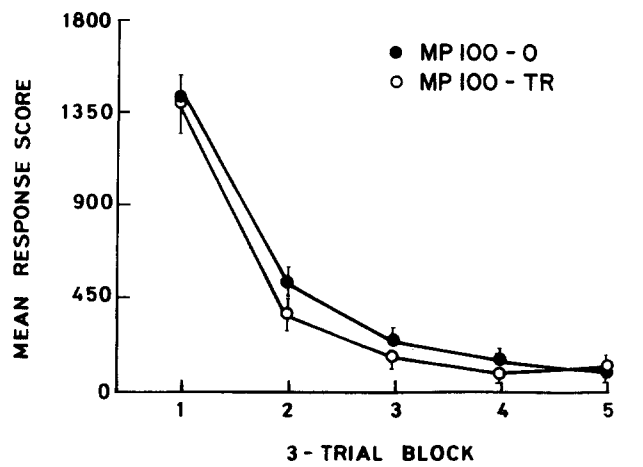

FIG. 5. Performances of 100 morphine μg/g-injected groups during a 15-trial *testing* session. Mean response score as in Fig. 4. The MP100-0 group had received no training after injection given 30 min before. The MP100-TR group had received a 15-trial *training* session 30 min after injection. Retention interval: 4 hr 30 min.

ment 2) is that morphine doses affecting the escape response to a passing screen, 30 min postinjection, fail to impair nystagmus following a similar injection-test interval. The implications of this result are two-fold. 1) The hypothesis that inhibition of the response to a danger stimulus is induced by a central morphine action and not by a detrimental effect on visual and/or motor ability (13) receives reasonable support, provided that the afferents and efferents for the optokinetic response of the crab are identical to those of the escape response. Consequently, morphine treatment seems to be a proper method to study the purported role of a central endogenous opioid mechanism in habituation to a danger stimulus, since morphine central action most likely mimics the endogenous opioid action. 2) Whenever a drug alters the crab's habituation of the escape response to a visual stimulus, the optokinetic response may serve as an end point to test the reliability of predominant central action of the agent. Of course, a negligible effect on nystagmus is a necessary but not sufficient condition to support central drug action.

Maldonado *et al.* (13) hypothesize that an opioid mechanism is involved in the crab's short-term habituation to a danger stimulus. A body of evidence supports this assumption: thus, short-term habituation to a danger stimulus flattens the reactivity to a subsequent new stimulus, either a painful or other danger stimulus (18,21); naloxone pretreatment both slows the short-term habituation and abolishes its flattening posteffect (17, 18, 21); and morphine pretreatment produces a dose-dependent reduction, naloxone reversible, of the danger-induced response (13). Results of the present paper (Experiments 3 and 4) allow us to infer certain aspects of the putative endogenous opiate mechanism during short-term habituation. Indeed, results from Experiment 3 suggest that morphine action reduces the escape response to a moving screen by activation of a modulatory system, and those from Experiment 4 that such a modulatory action is achieved by reducing the magnitude of the danger that the stimulus normally signals.

ACKNOWLEDGEMENTS

This work was supported by the Consejo Nacional de Investigaciones Cientificas y Tecnicas de Argentina, grant PID 3-042100-85, by the Buenos Aires University, grant Ex-131, and by Fundacion Antorchas. The authors are indebted to Angel Vidal for technical assistance.

REFERENCES

1. Applewhite, B. P.; Gardner, F. T.; Lapan, E. Physiology of habituation learning in a protozoan. Ann. NY Acad. Sci. 31:842–849; 1969.
2. Brunner, D.; Maldonado, H. Habituation in the crab *Chasmagnathus granulatus*. Effect of morphine and naloxone. J. Comp. Physiol. [A] 162:687–694; 1988.
3. Curio, E. Funktionsweise und Stammesgeschichte des Flugfeinderknnens einiger Darwin-finken (Geospizinae). Zeit. Tierpsychol. 26: 394–487; 1969.
4. Davis, M.; File, S. E. Intrinsic and extrinsic mechanisms of habituation and sensitization: implications for the design and analysis of experiments. In: Peeke, H. V. S.; Petrinovich, L., eds. Habituation, sensitization and behavior. Orlando: Academic Press, Inc.; 1984: 287–323.
5. Davis, M.; Wagner, A. R. Habituation of startle response under incremental sequence of stimulus intensities. J. Comp. Physiol. Psychol. 67:486–492; 1967.
6. Fanselow, M. S. Opiate modulation of the active and inactive components of the postshock reaction: parallels between naloxone pretreatment and shock intensity. Behav. Neurosci. 98:269–277; 1984.
7. Fanselow, M. S. Conditioned fear-induced opiate analgesia: A competing motivational state theory of stress analgesia. In: Kelly, D. D., ed. Stress-induced analgesia. New York: The New York Academy of Sciences; 1986:40–54.
8. Hentschel, E.; Penzlin, H. Beeinflussung des putzverhaltens bei *Periplaneta americana* (L) durch Wundsetzung, Naloxon-Morphin- und Met-Enkephalingaben. Zool. Jb. Physiol. 86:361–370; 1982.
9. Horridge, G. A.; Sandeman, D. C. Nervous control of optokinetic responses in the crab *Carcinus*. Proc. R. Soc. (B) 161:216–246; 1964.
10. Lozada, M.; Romano, A.; Maldonado, H. Effect of morphine and naloxone on a defensive response of the crab *Chasmagnathus granulatus*. Pharmacol. Biochem. Behav. 30:635–640; 1987.
11. Lozada, M.; Romano, A.; Maldonado, H. Long-term habituation to a danger stimulus in the crab *Chasmagnathus granulatus*. Physiol. Behav. 47:35–41; 1990.
12. Lozada, M.; Romano, A.; Maldonado, H. Effect of interstimulus

interval length on short- and long-term habituation in the crab *Chasmagnathus*. Submitted.
13. Maldonado, H.; Romano, A.; Lozada, M. Opiate action on response level to danger stimulus in the crab *Chasmagnathus granulatus*. Behav. Neurosci. 103:1139–1143; 1989.
14. Nalbach, H. O.; Nalbach, G. Distribution of optokinetic sensitivity over the eye of crabs: its relation to habitat and possible role in flow-field analysis. J. Comp. Physiol. [A] 160:127–135; 1987.
15. Nunez, J. A.; Maldonado, H.; Miralto, A.; Balderrama, N. The stinging response of the honey bee. Effects of morphine, naloxone and some opioid peptides. Pharmacol. Biochem. Behav. 19:921–924; 1983.
16. Peeke, H. V. S.; Veno, A. Stimulus specificity of habituated aggression in the three-spined sticklebacks (*Gasterosteus acualeatus*). Zeit. Tierpsychol. 40:53–58; 1976.
17. Romano, A.; Lozada, M.; Maldonado, H. Effect of naloxone pretreatment on habituation in the crab *Chasmagnathus granulatus*. Behav. Neural Biol. 53:113–122; 1990.
18. Romano, A.; Lozada, M.; Maldonado, H. Paradoxal effect of the retention interval length on long-term habituation in the crab *Chasmagnathus*. Submitted.
19. Sandeman, D. C. Compensatory eye movement in crabs. In: Hoyle, G., ed. Identified neurons and behaviour of arthropods. New York: Plenum Press; 1977:131–147.
20. Shone, H. Agonistic and sexual display in aquatic and semi-terrestrial brachyuran crabs. Am. Zool. 8:641–654; 1968.
21. Valeggia, C.; Fernandez-Duque, E.; Maldonado, H. Danger stimulus-induced analgesia in the crab *Chasmagnathus granulatus*. Brain Res. 481:304–308; 1989.
22. Warren, P. H.; Ison, J. R. Selective action of morphine on reflex expression to nociceptive stimulation in the rat: A contribution to the assessment of analgesia. Pharmacol. Biochem. Behav. 16:869–874; 1982.
23. Wright, H. O. Visual displays in brachyuran crabs: field and laboratory studies. Am. Zool. 8:655–665; 1968.
24. Zabala, N. A.; Miralto, A.; Maldonado, H.; Nunez, J. A.; Jaffe, K.; Calderon, L. de C. Opiate receptor in praying mantis: Effect of morphine and naloxone. Pharmacol. Biochem. Behav. 20:683–687; 1984.