Pergamon

Pharmacology Biochemistry and Behavior, Vol. 51, Nos. 2/3, pp. 223-229, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00325-4

Ethanol Affects Context Memory and Long-Term Habituation in the Crab *Chasmagnathus*

MIRIAM G. SARACO AND HECTOR MALDONADO[1]

Laboratorio de Fisiología del Comportamiento Animal, Facultad de Ciencias Exactas y Naturales, Depto de Biología, Pab 2, Buenos Aires University, (1428) Buenos Aires, Argentina

Received 29 April 1994

SARACO, M. G. AND H. MALDONADO. *Ethanol affects context memory and long-term habituation in the crab* Chasmagnathus. PHARMACOL BIOCHEM BEHAV **51**(2/3) 223-229, 1995. – A shadow moving overhead acts as a danger stimulus and elicits an escape response in the crab *Chasmagnathus granulatus* that habituates promptly and for a long period. The effect of acute ethanol treatment on this long-term memory was analyzed. A single injection of 0.01, 0.05, or 0.1 μg ethanol (ET)/g given 30 min before iterated presentation of a visual danger stimulus failed to affect short-term habituation. Posttraining ethanol (0.01 to 0.1 μg/g) produces a dose-dependent impairment of long-term habituation, but pretraining ethanol had no amnestic effect. However, a retention deficit confined to context memory was disclosed with both pre- and posttraining ethanol. Results from experiments with double injection (posttraining and pretesting injections) account for the retention impairment in terms of true amnesia (failure to acquire memory) but not due to state-dependence or retrieval deficit. The nonamnestic effect of pretraining ethanol upon long-term habituation is explained by a nonespecific depressing effect caused by interaction between iterative presentation of the danger stimulus and drug-induced internal state during training.

Crab Arthropoda Learning and memory Habituation Ethanol

WHEN a passing shadow (a danger stimulus) is presented to the crab *Chasmagnathus granulatus*, an escape response is elicited that habituates quickly after repeated presentation and for a long period (8,26). Research of our laboratory has been focused on defining the behavioral parameters of this process as well as on gaining insight into the mechanisms subserving the acquisition and retention of the habituated response (22, 31,32,37). Among these studies, several drugs with specific disrupting or facilitatory effect on long-term memory has been used as inhibitors of protein synthesis (cycloheximide and actinomicin-D) (26,27), GABA (36), and opioids (3,18,23,32,36). In this context, the present work is aimed at studying the effect of acute ethanol administration on *Chasmagnathus* long-term habituation.

According to recent studies with vertebrates, the effect of ethanol on the central nervous system would be explained in terms of alterations in receptor-mediated mechanisms, rather than by a nonspecific interaction with neural membranes, which used to be the currently accepted previous view (6). In fact, we have recently learned, for instance, that most of the pharmacological effects of ethanol can be related to the GABAergic transmission (20,34); or that acute ethanol treatment inhibits the dihydropyridine-sensitive Ca^{2+} channels (17) or opioids receptor binding (11,19).

This specific action of acute ethanol treatment would make it a potentially useful tool to study the mnemonic mechanisms, provided that an actual interference with memory were demonstrated. However, vertebrate experiments investigating ethanol effect have given mixed results.

As regards memory for aversive learning, ethanol-induced impairment was found in fishes (33) and rodents (3,4,12), although the latter was interpreted as resulting from posttraining addition to a complex stimulus rather than from a strengthening of memory traces (21). Other studies on rodents showed memory impairment (10) or no effect, despite administration of near lethal doses (29) or a reduced retention after seven daily injections of 3.6 g/kg.

Concerning no aversive tasks, posttraining ethanol has been reported to improve the correlation between training and testing latencies in a water-finding paradigm, but no actual

[1] To whom requests for reprints should be addressed.

reduction in test latencies was disclosed (25). An apparent memory impairment was also reported in a food-finding paradigm, but it proved to be a case of retrograde state dependency (5). On the other hand, recent experiments showed that a low dose of ethanol disrupted working memory in mice (24).

The effects of ethanol in crustaceans have been mainly studied as regards neurotransmission at the neuromuscular junction [e.g., (7,16,41)]. In contrast, no experiments concerning effect on memory retention have been reported, though behavioral changes induced by ethanol, as hyperexcitability, were described (16).

GENERAL METHOD

Animals

Animals were adult male *Chasmagnathus* crabs 2.6–2.9 cm across the carapace, weighing 15–16 g, collected from water less than 1 m deep in the rias (narrow coastal inlets) of San Clemente del Tuyú, Argentina, and transported to the laboratory where they were lodged in plastic tanks (35 × 48 × 27 cm) filled to 2 cm depth with water, at a density of 35 crabs per tank. Water used in tanks and other containers during experiments was prepared with hw-Marinex (Winex-Germany) (salinity 10–14 ‰, pH 7.4–7.6). The holding room was maintained on a 12 L : 12 D cycle (lights on 0700–1900 h). Animals were fed rabbit pellets (Nutrientes SA) every 3 days, and after feeding, the water was changed. Temperature of both holding and experimental rooms as well as the alley between them was maintained within a range of 19–24°C.

Experiments were carried out within the first week after the animal's arrival and between November and June (i.e., late spring, summer, and fall). Each crab was use in one experiment.

Apparatus

The apparatus is described in detail elsewhere (31). Briefly, the experimental unit was the actometer: a bowl-shaped plastic container with steep concave walls and a circular central flat floor 10 cm diameter, covered to a depth of 0.5 cm with water. The crab was lodged in the container that 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. An opaque rectangle screen (25 × 7.5 cm) could be moved horizontally across the upper border of the framework by a motor at an angular speed that allowed it to cover the entire opening in 2.3 s. Screen displacements provoked a crab's running response and, consequently, container oscillations. A stylus was centrally cemented to the bottom of the container and connected to a piezoelectric transducer. Container oscillations induced, through the transducer, electrical signals proportional to the velocity of the oscillations (9). Such signals were amplified, integrated during the recording time (9 s), and translated into numerical units ranging from zero to 3060 before being processed by computer. Thus, the scores were correlated proportionally to the velocity and number of the container oscillations recorded during 9 s. The amplification of the voltage changes was kept at such a gain that scores remained below 3060. The experimental room had 40 actometers, isolated from each other by partitions.

In order 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 noticeable uniformity of scores was obtained.

A computer was employed to program trial sequences, trial duration, and intertrial intervals, as well as to monitor experimental events.

Description of the Escape Response in the Actometer

The escape response in the actometer consists in the crab starting to run in an attempt to move away from the passing screen. However, because the steep concavity of the circular wall prevents the animal from climbing up, each running effort is confined to the flat center of the container in such a way that the escape response during a single trial looks like a series of flights from the center toward the base of the wall.

Experimental Procedure

A stimulation session consisted of a fixed number of trials given with 180-s intertrial intervals and preceded by 30 min of adaptation in the actometer. Each trial lasted 9 s and consisted of passing the screen four times over the actometer, recording the crab's activity during the entire trial time. Unless otherwise noted, the experimental design and procedure were as follows.

Crabs underwent two sessions per experiment, i.e., the training session (15 trials) and the testing session (1 trial) separated by a 48-h interval, that is, the longest period after which a robust retention was found when a 15-trial training is given (28). During the entire intersession interval, crabs were individually housed in plastic containers covered to a depth of 0.5 cm with water, and kept inside drawers dimly lighted. Four groups of 35–40 crabs each were run per experiment. Two groups were water injected (WA groups) and two were ethanol injected (ET groups), and in turn, one group of each pair was trained (TR group) and an other untrained (control group, CT group).

because the number of actometers was insufficient to run all groups of each experiment simultaneously, replications during the same day were necessary. An equal number of crabs per group was used in each replication with 40 individuals, but animals of a same group were placed in a different set of actometers each time. Thus, any potential effect of time of day and/or between-actometer differences was offset.

Before animals were injected to be used in 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 later time, they usually present unhealthy symptoms. No more than 10% of tested crabs were eliminated.

Injections

Distilled water (50 μl) (WA) or ethanol (ET) solution (50 μl) were given through the right side of the cephalotoraxic-abdominal membrane by means of a syringe fitted with a sleeve to control depth of penetration to 4 mm, thus ensuring that the injected solution was released roughly at the center of the pericardial sac. In pretraining experiments, injections were made 30 min before the first training trial, whereas in post-training experiments, injections were administered between 0–10 min after training, namely the time necessary to inject 40 crabs per replication. Ethanol was purchased from Raudo, Argentina.

Statistics

Retention was operationally defined as a significant difference between a trained group (WA-TR or ET-TR) and its

respective control group (WA-CT or ET-CT). Accordingly, ethanol is said to have amnestic effect when the difference between control and trained drug-injected crabs fails to reach the significance level, namely, when the null hypothesis H_0 proves to be true. Statistical analyses performed in our laboratory on this type of data, aimed at comparing the power of parametric and nonparametric tests, showed many instances in which a *U*-test had a greater power to reject H_0 than its parametric alternative, thus being likely a better test to avoid Type II errors. For that reason, and to avoid making assumptions concerning normality and homogeneity of variance as well as to increase the generality of our findings, the Mann-Whitney *U*-test was preferred ($\alpha = 0.05$).

Thus, the effect of ethanol was assessed by focusing the data analysis on testing scores. Rescorla (30) argued convincingly in favor of using this sort of analysis instead of a paired training-testing comparison, stressing the need to distinguish clearly between time of input (training session) and time of assessment (testing session).

Definitions

Throughout this article the following expressions are used with the meaning here defined. Short-term habituation refers to the response decrement within the training session; long-term habituation to a retention of a response decrement demonstrated in the testing session.

RESULTS

Pretraining Ethanol Does Not Affect Short-Term Habituation

Two groups of crabs, one preinjected with 0.1 μg ethanol/g (ET) and other with water (WA), underwent a 15-trial training session after a 30-min. adaptation.

Figure 1 shows the performances of ET and WA during the 15 training trials. The close resemblance of the curves is manifest, thus suggesting that the preinjection of ethanol has no effect on the short-term habituation of the iterated stimulus. A 2 × 15 ANOVA of repeated-measurements performed on these data disclosed no significant main effect, $F = 0.13$, nor interaction, $F = 0.78$. A similar picture was obtained with 0.01 or 0.05 μg/g. No overt symptoms of hyperexcitability appeared during the training session (1 h 15 min) with any of these doses.

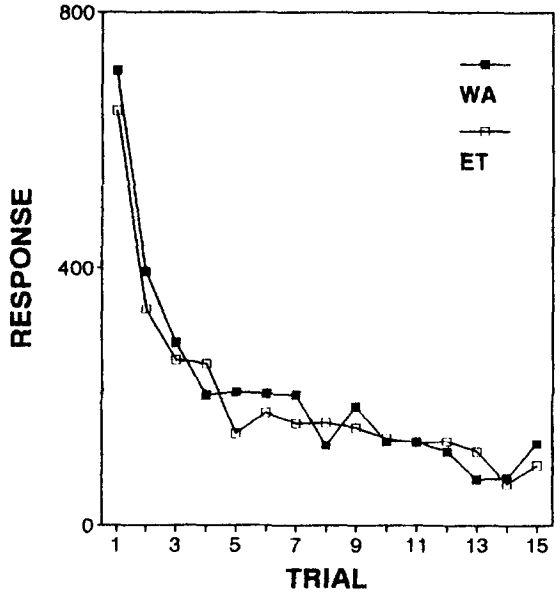

FIG. 1. Effect of ethanol on short-term habituation. Black squares stand for the distilled water injected group (WA); white squares for the group injected with 0.1 μg ethanol/g (ET). Ordinates: mean of escape response scores. Abscissae: trials of 9 s each, 3-min intertrial.

However, crabs given doses between 0.5 and 1.0 μg/g showed, for roughly 30–40 min, episodes of freezing with legs and claws extension, as well as trembling and disordered movements of chelae. Twenty-four hours after injection appendage losses, a phenomenon termed autotomy (15) and a growing number of deaths correlated with increasing dose were detected (5 out of 80 for 0.5 μg ethanol/g; 30 out of 80 for 1 μg/g).

Thus, 0.01–0.1 μg ethanol/g administered 30 min before training seems not to affect either short-term habituation or response level to danger stimulus.

Pretraining Ethanol Does Not Impair Long-Term Habituation

A long series of results from previous experiments at our laboratory indicates that a 15-trial training session ensures a robust retention for 48 h [e.g., (28)]. Therefore, such amount of training and intersession interval was chosen for the following experiments.

An equal number of crabs was randomly assigned to four groups in a 2 × 2 factorial design, the factors being drug (WA, or ET: 0.1 μg ethanol/g; both injected 30 min before training), and training (CT: control, no training; or TR: 15-trial training); so that groups were: WA-CT, WA-TR, ET-CT, and ET-TR. Both control groups (WA-CT and ET-CT) remained in actometers during the time corresponding to the training session (1 h 15 min), but without being stimulated by the passing shadow.

The statistical analysis on data corresponding to the testing trial (Fig. 2a) disclosed a significant difference for WA-CT vs. WA-TR and for ET-CT vs. ET-TR, but no significant difference between control groups (WA-CT vs. ET-CT) nor between trained groups (WA-TR vs. ET-TR).

A similar picture of results was obtained in an identical experiment with 0.01 μg/g (Fig. 2b).

Therefore, the 48 h-retention of the habituated response seems to be impervious to these doses of preinjected ethanol.

Pretraining Ethanol Impairs Context Memory

Memory of the context has been demonstrated to have a critical role in the *Chasmagnathus* long-term habituation, and an interpretation close to the associative theory of habituation (38,39,40) was offered (37).

On this account, the following experiment was performed to test the preinjected ethanol effect on the contextual memory. According to a strictly associative interpretation, no amnestic effect should be expected, because no effect was found on long-term habituation.

One hundred and sixty crabs randomly distributed in two groups of 40 each: the same-context group (SAM) and the different-context group (DIF). SAM animals remained in the actometer for 90 min, namely, for the total time crabs stay in the apparatus during a usual 15-trial training, but without being confronted with the passing shadow, and given one testing trial in the actometer after 48 h. DIF animals were individ-

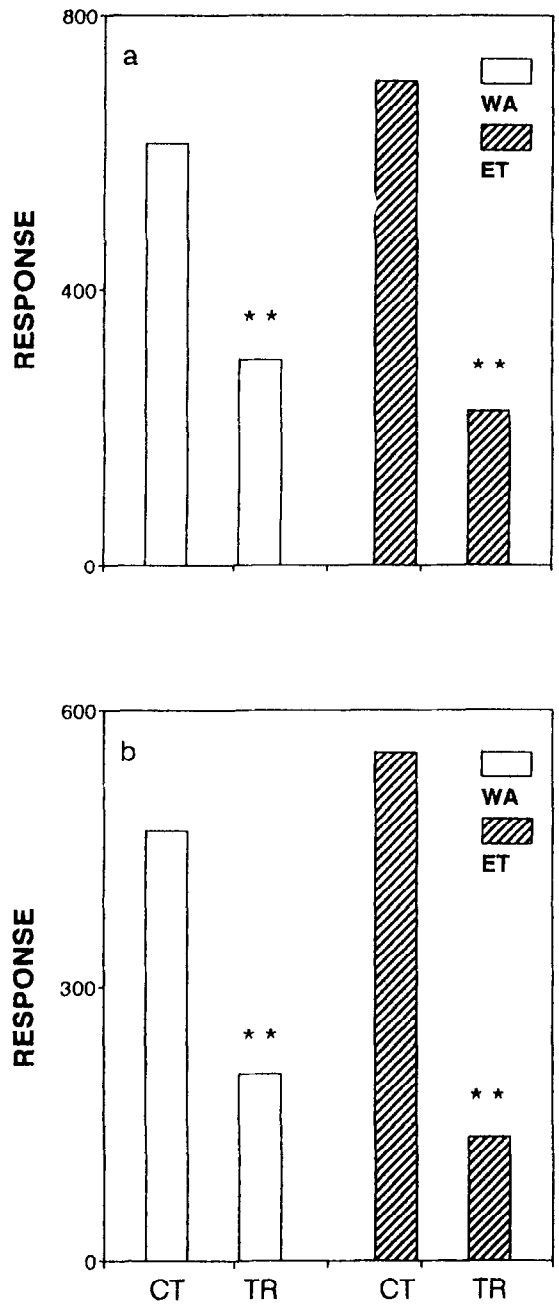

FIG. 2. Effect of pretraining ethanol on long-term habituation tested at a 48-h time interval. (a) ET groups injected with 0.1 μg/g; (b) with 0.01 μg/g. White bars stands for groups water injected 30 min before training (WA groups); striped bars for ethanol injected groups (ET groups). Ordinates: median of the testing-trial scores. Abscissae: CT, a control group (WA-CT or ET-CT); TR, a trained group (WA-TR or ET-TR). Mann-Whitney test: **stands for $p < 0.01$ in comparisons between WA groups [(a) z = 3.34; (b) z = 4.07] or ET groups [(a) z = 4.66; (b) z = 3.47].

ually housed for 90 min in dimly lighted boxes outside the apparatus, and tested in the actometers after a 48-h interval. Half of the crabs in each group were preinjected with WA and the other half with 0.1 μg/g of ethanol. Thus, four subgroups were formed and named WA-SAM, WA-DIF, ET-SAM, and ET-DIF.

Figure 3 illustrates the performances at testing. The response level of WA-DIF was significantly higher than that of WA-SAM, a result in keeping with previous reports (26,37). In fact, it has been demonstrated that crabs receiving prior exposure to the actometer exhibit lower response at testing than a group preexposed to a wholly dissimilar context. The significant difference between WA-SAM and WA-DIF stands for retention of contextual memory.

On the other hand, no significant difference was disclosed between ET-SAM and ET-DIF whose response levels were, in turn, similar to that of the WA-DIF.

Thus, whereas 0.1 μg/g of preinjected ethanol showed no amnestic effect on long-term habituation, the same dose impairs the contextual memory.

This result is at variance with the associative theory of habituation and close similar to that obtained in *Chasmagnathus* when cycloheximide was injected before training (26). However, recent results disclosed a clear-cut amnestic effect on both the contextual memory and long-term habituation when an inhibitor of protein synthesis, either cycloheximide or actinomicin-D, was posttraining administered (26,27). On this account, the absence of amnestic effect in preinjected trained crabs was explained in terms of a nonspecific depressing effect on responsiveness, due to the interaction between a drug-induced internal state and the iterated presentation of the danger stimulus. Such a depressing effect was shown to disappear when crabs were tested 72 h after training (26).

Therefore, the following experiments were aimed at testing the effect of posttraining ethanol.

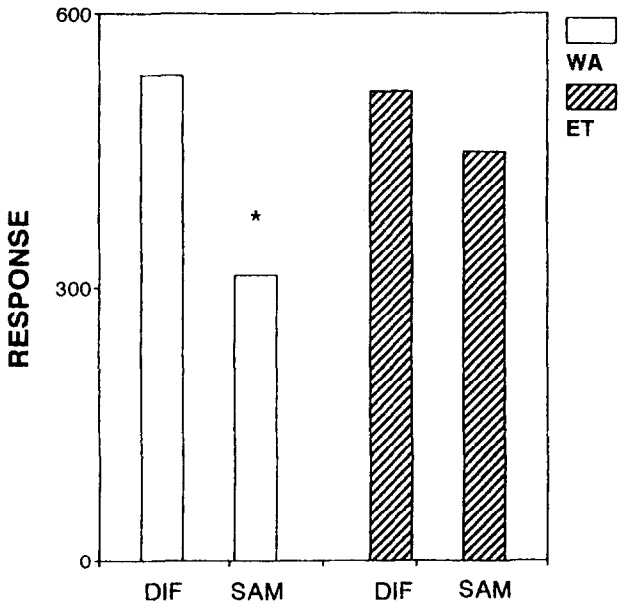

FIG. 3. Context memory. Effect of pretraining ethanol (0.1 μg/g) when tested 48 h after training. White bars stands for groups water injected 30 min before training (WA groups); striped bars for ethanol injected groups (ET groups). Ordinates: median of the testing-trial scores per group. Abscissae: DIF, a group kept in dimly lighted containers during training and tested in the actometer (WA-DIF or ET-DIF); SAM, a group kept in actometers at both training and testing (WA-SAM or ET-SAM). Mann-Whitney test: *stands for $p < 0.05$ in comparisons between WA groups (z = 1.75).

Effect of Posttraining Ethanol on Long-Term Habituation and Context Memory

Experiments of this section had the above experimental design except that ethanol was administered immediately after training.

Figure 4 displays results corresponding to the testing trial of three long-term habituation experiments with doses of 0.1, 0.05, and 0.01 μg/g of ethanol. A significant difference was found between WA-injected groups (WA-CT vs. WA-TR) in all the three cases. Groups injected with either 0.1 or 0.05 μg/g (ET-CT vs. ET-TR) failed to show a significant difference and, in addition, the response value of ET-CT was higher than that of ET-TR in both cases (Fig. 4a and b). On the contrary, groups given 0.01 μg ethanol/g revealed a difference between ET-CT and ET-TR similar to that shown by controls groups.

Thus, results suggest a dose-dependent impairment of long-term habituation induced by posttraining ethanol.

The effect of 0.1 μg/g on the contextual memory is illustrated in Fig. 5, where results corresponding to the testing trial are shown. As usual, the responsiveness of WA-DIF was significantly higher than that of WA-SAM, while no significant difference was disclosed between ET-SAM and ET-DIF whose response levels were, in turn, similar to that of the WA-DIF.

In summary, doses of 0.1 μg ethanol/g given after training show an amnestic effect both on long-term habituation and on the contextual memory.

A further experiment was conducted to address the possibility that the above effect of ethanol might be an instance of state dependence (21). If this were the case, retention should not be impaired when ethanol is given in association with both training and testing.

Four groups of animals as those corresponding to Fig. 4a were made up but receiving water or ethanol both immediately after training and 30 min before testing, so that they were named WA.WA-CT, WA.WA-TR, ET.ET-CT, and ET.ET-TR.

Results displayed in Fig. 6 were close similar to those obtained with a single posttraining injection (Fig. 4a): a significant difference for WA.WA-CT vs. WA.WA.-TR but no significant difference between ET.ET-CT and ET.ET-TR groups or between control groups (WA.WA-CT vs. ET.ET-CT).

Therefore, the impairment or long-term habituation induced by posttraining ethanol seems due to true amnesia (failure to form memory) but not to state dependence, because recall was not reinstated despite a return to the same drug state as that of the storage phase.

DISCUSSION

Doses equal to or lower than 0.1 μg ethanol/g, administered 30 min before training, have no effect either on the escape response level or on short-term habituation or on the 48-h retention of the habituated response, but impairs contextual memory. On the other hand, 0.1 μg ethanol/g injected after training impairs both long-term habituation and memory of context.

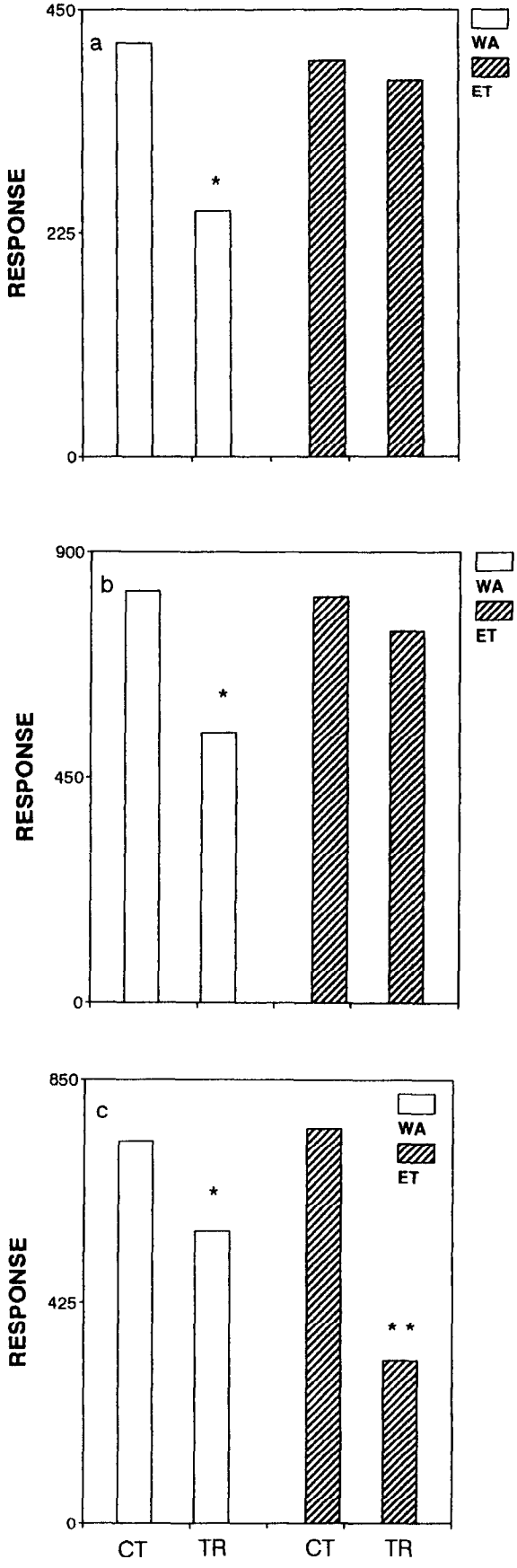

FIG. 4. Effect of posttraining ethanol on long-term habituation tested at a 48-h time interval. (a) ET groups injected with 0.1 μg/g; (b) with 0.05 μg/g; (c) with 0.01 μg/g. Mann-Whitney test: *stands for $p < 0.05$ and ** for $p < 0.01$ in comparisons between WA groups [(a) $z = 2.48$; (b) $z = 1.92$; (c) $z = 1.77$] or between ET groups [(c) $z = 4.24$]. Other symbols as in Fig. 2.

The memory deficit cannot be explained in terms of nonamnestic effects. First, no significant difference was found throughout between context control groups (WA-DIF vs. ET-DIF), so that retention impairment is not attributable to a generalized enhancing effect of the drug. Second, amnesia is expressed by a positive act on the part of the trained crabs (an increase in reactivity of the trained ET groups), so that ethanol effect cannot be accounted for by an association between a drug-aversive effect and the experimental situation. Lastly, the exclusion of "state-dependent" learning as alternative explanation (Fig. 6) suggest that the amnestic effect involves a failure to form memory but not a deficit in the ability to retrieve information.

The fact that ethanol disrupts long-term habituation to the danger stimulus when injected after but not before training might be explained as follows. Pretraining ethanol does not affect acquisition and, in addition, because the drug action lasts for a short time, neither the memory retention is affected; on the contrary, the action of posttraining ethanol incides on the storage phase, thus disrupting the long-term habituation. However, as the habituation to the visual danger stimulus is context specific (35), and as ethanol disrupts contextual memory either when pre- or posttraining injected, an alternative explanation is favored. Namely, the pretraining ethanol fails to impair long-term habituation due apparently to a nonspecific depressing effect upon the escape response, caused by interaction during training between the iterative presentation of the danger stimulus and the ethanol-induced internal state. A similar explanation is offered for a like pattern of results

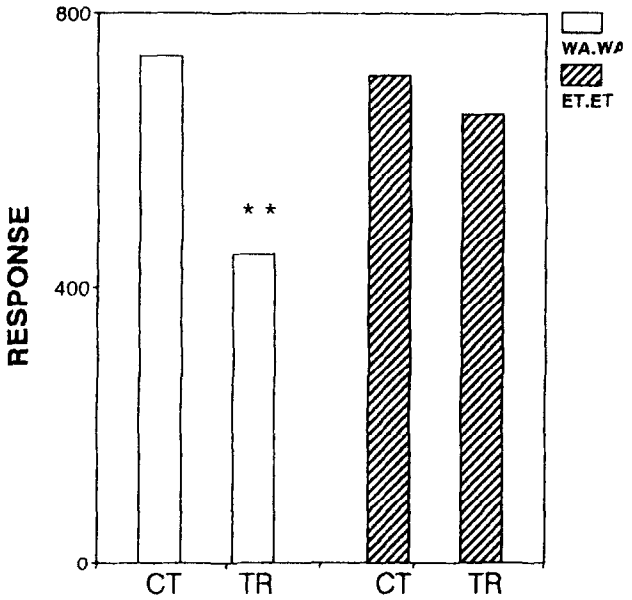

FIG. 6. Effect of posttraining and pretesting ethanol on long-term habituation when tested 48 h after training. White bars stands for groups who were water injected immediately after training and 30 min before testing (WA.WA groups); striped bars for groups who were injected with 0.1 μg/g of ethanol immediately after training and 30 min before testing (ET.ET groups). Mann-Whitney test: **stands for $p < 0.01$ in comparisons between WA.WA groups [z = 2.76]. Other symbols as in Fig. 2.

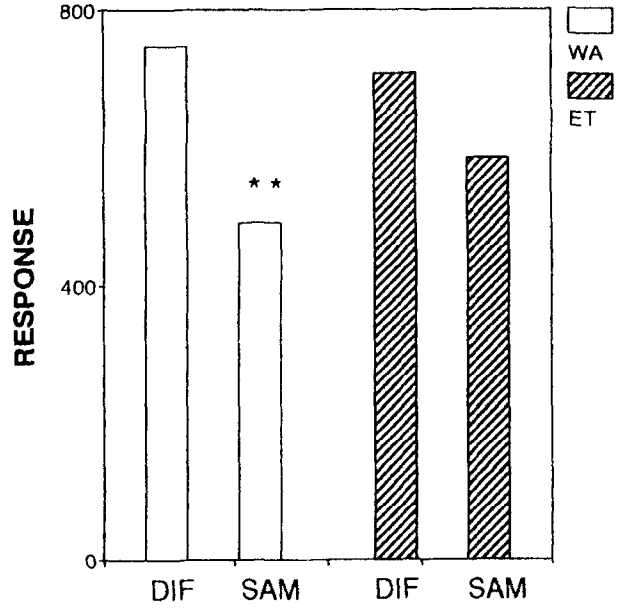

FIG. 5. Context memory. Effect of posttraining ethanol (0.1 μg/g) when tested 48 h after training. Mann-Whitney test: **stands for $p < 0.05$ in comparisons between WA groups [z = 1.95]. Other symbols as in Fig. 3.

obtained when *Chasmagnathus* is injected inhibitors of the protein synthesis (26,27).

An additional point is worth mentioning. The ethanol doses that impair memory in *Chasmagnathus* are remarkably lower than those required to show some effect upon retention by systemic injection in intact vertebrates. In fact, the *Chasmagnathus* doses are 0.05 and 0.1 μg/g, while those of vertebrates are about 1.0–2.0 g/kg [e.g., (5,13)]. A similar difference is shown with other drugs [e.g., inhibitors of protein synthesis (26,27)], and a possible explanation could be the fact that no endothelial blood-brain barrier like that of vertebrates exists in crabs (1,2).

To sum up, results in the present article indicating that ethanol disrupts long-term memory, as well as recent studies with vertebrates suggesting that ethanol would interfer with intracellular signalling processes, makes this amnesic agent a potentially useful tool for studying mechanistic aspects of long-term habituation.

ACKNOWLEDGEMENTS

This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina, Grant PID 216-89, by the University of Buenos Aires, Grant Ex-134, and by Fundación Antorchas. The authors are deeply indebted to Angel Vidal for technical assistance.

REFERENCES

1. Abbott, N. J. Comparative physiology of the blood-brain barrier. In: Bradbury, M. W. B., ed. Physiology and pharmacology of the blood-brain barrier. Berlin: Springer Verlag; 1992:371-396.
2. Abbott, J. Absence of blood-barrier in a crustacean, *Carcinus maenas* L. Nature 225:291-293; 1970.
3. Alkana, R. L. Alcohol and memory: Facilitation by posttraining

administration in mice. Subst. Alcohol Actions/Misuse 1:173–180; 1980.
4. Alkana, R. L.; Parker, E. S. Memory facilitation by posttraining injection of ethanol. Psycopharmacology (Berlin) 66:117–119; 1979.
5. Babbini, M.; Jones, B. L.; Alkana, R. L. Effects of posttraining ethanol on group housing upon memory of an appetitive task in mice. Behav. Neural Biol. 56:32–42; 1991.
6. Blum, K. Molecular mechanisms of intracellular ethanol action. Experientia 45:406; 1989.
7. Blundon, J. A.; Bittner, G. D. Effects of ethanol and other drugs on excitatory and inhibitory neurotransmission in the crayfish. J. Neurophysiol. 67:576–587; 1992.
8. Brunner, D.; Maldonado, H. Habituation in the crab *Chasmagnathus granulatus*: Effect of morphine and naloxone. J. Comp. Physiol. [A] 162:687–694; 1988.
9. Cady, W. G. Piezoelectricity. New York: Dover; 1964.
10. Castellano, C.; Pavone, F. Effects of ethanol on passive avoidance behavior in the mouse: Involvement of GABAergic mechanisms. Pharmacol. Biochem. Behav. 29:321–324; 1988.
11. Charness, M. E. Ethanol and opioid receptor signaling. Experientia 45:418–427; 1989.
12. Colbern, D. L.; Shareck, P.; Zimmerman, E. G. The effect of home or novel environment on the facilitation of passive avoidance by posttraining ethanol. Behav. Neural Biol. 46:1–12; 1986.
13. Criswell, H. E.; Breese, G. R. Similar effects of ethanol and flumazenil on acquisition of a shuttle-box avoidance response during withdrawal from chronic ethanol treatment. Br. J. Pharmacol. 110:753–760; 1993.
14. Fernandez-Duque, E.; Vallegia, C.; Maldonado, H. Multitrial inhibitory avoidance learning in the crab *Chasmagnathus*. Behav. Neural Biol. 57:189–197; 1992.
15. Fredericq, L. Sur l'autotomie ou mutilation par voie reflexe comme moyen de defense chez les animaux. Arch. Zool. Exp. Gen. 1:413–426; 1883.
16. Friedman, R. N.; Bittner, G. D.; Blundon, J. A. Electrophysiological and behavioral effects on crayfish. J. Pharmacol. Exp. Ther. 246:125–131; 1988.
17. Ghandi, C. R.; Ross, D. H. Influence of ethanol on calcium, inositol phospholipids and intracellular signaling mechanisms. Experientia 45:407–413; 1989.
18. Godoy, A.; Maldonado, H. Modulation of the escape response by [D-Ala^2]Met-enkephalin in the crab *Chasmagnathus*. Pharmacol. Biochem. Behav. 50:445–451; 1994.
19. Hiller, J. M.; Angel, L. M.; Simon, E. J. Characterization of the selective inhibition of the delta subclass of opioid binding sites by alcohol. Mol. Pharmacol. 25:249–255; 1984.
20. Hunt, W. A. The effect of ethanol on GABAergic transmission. Neurosci. Biobehav. 7:87–95; 1983.
21. Izquierdo, I. Different forms of posttraining memory processing. Behav. Neural Biol. 51:171–202; 1989.
22. Lozada, M.; Romano, A.; Maldonado, H. Long term habituation to a danger stimulus in the crab *Chasmagnathus granulatus*. Physiol. Behav. 47:35–41; 1990.
23. Maldonado, H.; Romano, A.; Lozada, M. Opioid action on response level to a danger stimulus in the crab *Chasmagnathus granulatus*. Behav. Neurosci. 103:1139–1143; 1989.
24. Melchor, C. L.; Glasky, A. J.; Ritzmann, R. E. A low dose of ethanol impairs working memory in mice in a win-shift foraging paradigm. Alcohol 10:491–493; 1993.
25. Melia, K. F.; Ehlers, C. L.; Le Brun, C. J.; Koob, L. Post learning ethanol effects on a water-finding task in rats. Pharmacol. Biochem. Behav. 24:1813–1815; 1986.
26. Pedreira, E.; Dimant, B.; Quesada-Allue, L.; Maldonado H. Cycloheximide affects long-term habituation and context memory in the crab *Chasmagnathus*. Pharmacol. Biochem. Behav. (in press).
27. Pedreira, E.; Dimant, B.; Maldonado, H. disruption of context memory and long-term habituation in the crab *Chasmagnathus* by inhibitors of the protein synthesis. (Submitted for publication).
28. Pereyra, P.; de la Iglesia, H.; Maldonado, H. Twelve hours of difference between day-time of training and testing impairs long-term habituation in the crab *Chasmagnathus*. (Submitted for publication).
29. Prado de Carvalho, L.; Vendite, D. A.; Izquierdo I. A near lethal dose of ethanol, given intraperitoneally, does not affect memory consolidation of two different avoidance tasks. Psychopharmacology (Berlin) 59:71–74; 1978.
30. Rescorla, R. A. Behavioral studies of pavlovian conditioning. Annu. Rev. Neurosci. 1:329–352; 1988.
31. Romano, A.; Lozada, M.; Maldonado, H. Effect of naloxone pretreatment on habituation in the crab *Chasmagnathus granulatus*. Behav. Neural Biol. 53:113–122; 1990.
32. Romano, A.; Lozada, M.; Maldonado, H. Non habituation processes affect stimulus specificity of the response-habituation in the crab *Chasmagnathus granulatus*. Behav. Neurosci. 105:542–552; 1991.
33. Scobie, S. R.; Bliss, D. K. Ethyl alcohol: Relationships to memory for aversive learning in Goldfish (*Carassius auratus*). J. Comp. Physiol. Psychol. 86:867–874; 1974.
34. Ticku, M. K. Ethanol and the benzodiazepine-GABA receptor-ionophore complex. Experientia 45:413–418; 1989.
35. Tomsic, D.; Maldonado H. Central effect of morphine pretreatment in short and long-term habituation to a danger stimulus in the crab *Chasmagnathus*. Pharmacol. Biochem. Behav. 36:787–793; 1990.
36. Tomsic, D.; Maldonado, H.; Rakitin, A. Morphine and GABA: Effects on perception, escape response and long-term habituation to a danger stimulus in the crab *Chasmagnathus*. Brain Res. Bull. 26:699–706; 1991.
37. Tomsic, D.; Massoni, V.; Maldonado, H. Habituation—A danger stimulus in two semiterrestrial crabs. Ontogenic, ecology and opioid modulation correlates. J. Comp. Physiol. 173:621–633; 1993.
38. Wagner, A. R. Priming in STM: An information processing mechanism for self-generated or retrieval-generated depression in performance. In: Tighe, T. S.; Leaton, R. N., eds. Habituation: Perspectives from child development, animal behavior and neurophysiology. Hillsdale, NJ: Lawrence Erbaum Associates; 1976: 95–128.
39. Wagner, A. R. Expectancies and the priming of STM. In: Hulse, S. H.; Fowler, H.; Honing, W. K., eds. Cognitive processes in animal behavior. Hillsdale, NJ: Lawrence Erlbaum Associates; 1978:177–209.
40. Wagner, A. R. Habituation and memory. In: Dickinson, A.; Bookes, R. A., eds. Mechanisms of learning and motivation: A memorial volume for Jerzy Konorski. Hillsdale, NJ: Lawrence Erlbaum Associates; 1979:53–82.
41. Watchel, R. E. Aliphatic alcohols increase the decay rate of glutamate-activated currents at the crayfish neuromuscular junction. Br. J. Pharmacol. 83:393–397; 1984.