Physiology & Behavior, Vol. 43, pp. 317–320. Copyright © Pergamon Press plc, 1988. Printed in the U.S.A. 0031-9384/88 $3.00 + .00

Passive Avoidance Learning in the Crab *Chasmagnathus granulatus*

ALICIA DENTI, BEATRIZ DIMANT AND HECTOR MALDONADO

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

Received 22 April 1987

DENTI, A., B. DIMANT AND H. MALDONADO. *Passive avoidance learning in the crab* Chasmagnathus granulatus. PHYSIOL BEHAV **43**(3) 317–320, 1988.—Male crabs *Chasmagnathus granulatus* were trained by means of a method similar to the standard inhibitory avoidance technique widely used in vertebrates. Each crab was placed in the dark compartment (DC) of a double-chamber device, allowed to move towards the light compartment (LC) and latency to enter measured. Experimental crabs received a shock in LC, but controls were not punished. After 1 min, both experimental and control crabs were free to return to DC. On completion of 1, 2, 3 or 24 hr intertrial interval in DC a retention test was administered and latency to enter LC was measured. A single trial was proven enough to establish a LC-shock association that was detected up to 3 hr later, but no retention was proved after 24 hr. Memory was disrupted when crabs were removed from the apparatus during the 3 hr intertrial interval. Similarities and differences between the passive avoidance method used with crabs and that used with vertebrates are discussed.

Avoidance learning One trial learning Crustacean Arthropods

A great deal of work has been carried out to elucidate the function of opiates as memory modulatory agents. It must be pointed out, however, that such research was almost entirely performed with vertebrates, all the more remarkable given the greater simplicity of invertebrate nervous systems [16], and the fact that arthropods are proving increasingly useful in studies of morphine-induced analgesia [10, 15, 19, 28]. Besides, cells containing opioid-like peptides have been detected in invertebrate nervous systems [4, 5, 8, 20, 21, 27]. Our laboratory is currently developing a project intended to study opiate action on memory in an arthropod that has been proven to be a good assay model for morphine-induced analgesia, the crab *Chasmagnathus granulatus* [14]. The project includes various learning paradigms, one of which, the habituation paradigm, has been recently used successfully [2]. The purpose of the present paper is to assay on a crustacean an inhibitory avoidance paradigm similar to that used for vertebrates. This technique was chosen since it is a major procedure employed to study the effect of pharmacological agents and other treatments on vertebrate learning and memory [7]. The popularity of passive avoidance training reflects several advantages, including three of its most remarkable features: the response is quickly learned (one trial learning), the training trial requires no pretraining, and memory is stable for a substantial time after training.

Previous papers have presented evidence of rapid and stable taste-aversion learning by several invertebrates [1, 12, 23], and of shock avoidance learning by cockroaches [3, 13, 17, 24, 25]. However, training with a method similar to the standard passive avoidance technique [11] has not been reported for animals other than vertebrates.

METHOD

Animals

The animals were adult male *Chasmagnathus granulatus* crabs 3.0–3.2 cm across the carapace, collected from water less than 1 m deep in the rias of San Clemente del Tuyu, Argentina, and transported to the laboratory, where they were lodged in glass tanks (35×48×27 cm) with 1–2 cm depth of water and walls painted black. Both holding-room and experimental-room were kept at constant temperature (18–20°C). Each crab was used in only one experiment.

Experimental Device

Each *training unit* consisted of a plastic box (25×25×15 cm) divided by a central partition in two compartments of the same size: *the dark compartment* (DC) and *the light compartment* (LC). DC had its walls painted black and a removable roof that prevented stray light from entering. LC was illuminated by a 25 W lamp and had its walls painted white. Both compartments were fitted with two stainless steel plates (11×2 cm) placed on opposite walls other than the partition, close to the floor, and connected to the output of an electric stimulator. A sliding door in the central partition allowed a crab to pass from DC to LC or vice-versa. The floor of each compartment was covered by a 0.5 cm deep layer of water, and a 1.0 cm high door threshold avoided inter-mixing. There were 15 training units in an isolated room at constant temperature (18–20°C). A mirror over each training unit allowed an experimenter to observe from a suitable place the inside of the 15 LCs, simultaneously.

Experimental Procedure

Each crab was moved from the holding-room to DC of one training unit around noon time.

Training trial. After 1 hour of *adaptation interval* in DC, the sliding door was raised and the *first latency* L1 to enter the LC was measured, i.e., the period elapsing from the instant the door was lifted till the crab was entirely in LC. The door was then lowered to prevent the animal from moving back into DC, and a shock (50 Hz, 16 V, 4 sec) was delivered. After 1 min, the door was raised again. If the animal failed to return at once to DC, it was induced to move through the door by gentle prodding.

Intertrial interval. No soomer had the animal entered DC the door was lowered and the *intertrial interval* began. The latter was 1, 2, 3 or 24 hr long.

Test trial. At the end of the intertrial interval the door was raised and the *second latency* L2 was calculated, i.e., the period from the instant the door was lifted till the crab wholly entered LC.

L1 and L2 were recorded by means of 15 stop-watches, i.e., one stop-watch per experimental unit. Slow displacements of the crabs allowed a single experimenter to record latency times readily and reliably. Cutoff scores of 900 sec were used for both latencies. Less than 15% of the animals failed to enter LC within 900 sec after the adaptation interval and were discarded.

Experimental Design

Pertinent details are given when presenting results.

In order to estimate a possible LC-shock association, the difference between the first and second latency values for each crab was evaluated as *percentage latency change* (PLC): $(L2-L1/900-L1)\times 100$ when $L2>L1$, i.e., percentage of maximum possible increase, and $(L2-L1/L1)\times 100$ when $L2<L1$, i.e., percentage of maximum possible decrease. When $L2=L1$, a zero value resulted.

RESULTS AND DISCUSSION

Experiment 1

One hundred and eighty animals were randomly assigned to 3 sets of 2 groups each, 30 crabs per group: set 1: 1H-EXPERIMENTAL and 1H-CONTROL groups, with 1 hour of intertrial interval; set 2: 2H-EXPERIMENTAL and 2H-CONTROL groups, 2 hours; and set 3: 3H-EXPERIMENTAL and 3H-CONTROL groups, 3 hours. Experimental groups were given a shock in LC during the training trial; control groups received no shock. Groups belonging to the same set were run together.

Figure 1 depicts mean PLCs. An ANOVA was performed on the 180 PLCs considering two factors: treatment (2 levels: control and experimental) and intertrial interval (3 levels: 1, 2 and 3 hr). The 2 factors involved were shown to cause significant difference between levels [*treatment*, $F(1,174)=31.12$, $p<0.05$; *intertrial interval*, $F(2,174)=3.8$, $p<0.05$]. In contrast, there was no significant interaction between factors ($F=0.11$). Two linear regressions, one performed on results with controls and the other on results with experimental animals, disclosed similar slope coefficients (Fig. 1).

Three conclusions may be drawn from this analysis. Firstly, a single training trial was enough to establish an association between LC and shock, which was retained for at least three hours. Secondly, lengthening of the intertrial interval produced significant reduction in PLCs both in control and experimental animals, i.e., the greater the interval, the greater the haste of animals to leave DC. Thirdly, experimental animals presented a PLC-interval curve displaced parallel to that of the controls, a shift attributable to their noxious experience in LC during the training trial.

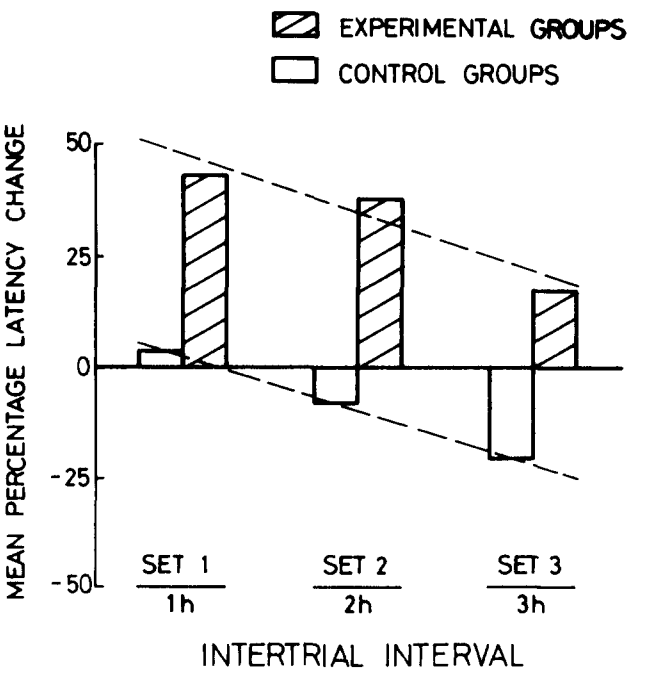

FIG. 1. Percentage latency change (PLC) vs. intertrial interval. Upper dashed line: linear regression by pairing PLCs of experimental groups with intertrial intervals. Lower dashed line: linear regression by pairing PLCs of control groups with intertrial intervals.

However, two alternative explanations must be considered before accepting the foregoing conclusions. (1) An electric shock produces profound alterations over a whole range of behaviorally important variables, mainly motivation and arousal [9,22], so that the purported avoidance learning could be a proactive consequence of the shock irrespective of compartment. (2) It is well known that many animals subjected to aversive stimuli or confronted with adverse situations release stress odors [6, 18, 26]. Therefore, it might be argued that the greater PLCs of the experimental groups versus controls should be attributed to scent released in LC during the training trial rather than to LC-shock association.

These alternative explanations will be analyzed along with the results of the following experiment.

Experiment 2

Sixty crabs were randomly assigned to 2 groups of 30 animals each: on one hand, the 1H-INTERVAL CONTROL group, with 1 hr of intertrial interval but no shock, i.e., as the 1H-CONTROL group of Experiment 1; and on the other hand, the 1H-INTERVAL SHOCKED group that received the shock at 30 min of the 1 hr intertrial interval, unlike the 1H-EXPERIMENTAL group of Experiment 1 which was given the shock in LC during the training trial. The mean PLC of the 1H-INTERVAL CONTROL (3.0, SE: 3.9) was not significantly different from that of the 1H-INTERVAL SHOCKED (2.6, SE: 3.4).

Another 70 crabs were divided into 2 groups: the ADAPTATION SHOCK (n=37) group that received one DC shock at min 30 of the 1 hr adaptation interval, and the ADAPTATION CONTROL (n=33) with also 1 hr of adaptation interval, but no shock. After the adaptation interval, the first latency values (L1) for both groups were measured. No significant difference between mean L1s was found (mean for the 1H-ADAPTATION CONTROL: 210.5, SE: 32.0; mean for the 1H-ADAPTATION SHOCK: 221, SE: 38.0).

Thus, neither the PLCs nor the first latency values were modified by a shock unassociated with LC. This result makes the first alternative explanation hardly tenable and, furthermore, proves to be at variance with a prediction stemming from the second alternative explanation. If the "scent" explanation were actually valid, greater haste should be expected in experimental animals to leave DC when the shock was delivered there. Thus, a lower mean PLC for the 1H-INTERVAL SHOCK versus controls, and also a lower mean L1 for the ADAPTATION SHOCK, would be obtained. However, results of Experiment 2 contradict such a prediction.

Experiment 3

Taking into account that 24 hr of intertrial interval is the most commonly used period for vertebrate inhibitory avoidance training, retention after such a period was tested in this experiment. Crabs were randomly assigned to one of two groups (n=30): the 24H-EXPERIMENTAL and 24H-CONTROL groups. Both groups had 24 hr intertrial interval and were run together, but whereas the former was given a shock during the training trial, the latter received no shock. Despite preliminary observations in our laboratory, animals of both groups proved healthy and active during the test trial. However, no significant intergroup difference was found regarding mean PLCs (mean for 24H-CONTROL: −13.4, SE: 8.4; mean for 24H-EXPERIMENTAL: 17.9, SE: 8.7).

Experiment 4

In order to test the effect of moving the animals to the holding-room during the intertrial interval, 60 crabs were randomly assigned to 2 groups of 30 animals each: the 2H+1H-EXPERIMENTAL and 2H+1H-CONTROL groups. The former group was given a shock in LC during the training trial, moved from the DC to their glass tank in the holding-room for 2 hours, then moved back to DC, and after 1 hr the test trial started. The corresponding controls received the same treatment but no shock was given. Thus, these groups were similar to the 3H-EXPERIMENTAL and 3H-CONTROL groups of Experiment 1, respectively, except regarding the modalities of the 3 hr intertrial interval. No significant intergroup difference was found regarding the mean PLCs (mean for 2H+1-CONTROL: −17.3, SE: 11.0; mean for 2H+1-EXPERIMENTAL: 0.5, SE: 8.6).

Comparison of this result with that obtained for the 3H-EXPERIMENTAL and 3H-CONTROL groups (Experiment 1) suggests that the LC-shock association established during the training trial was disrupted by a retroactive interference, i.e., by removing animals from the apparatus.

GENERAL DISCUSSION

Results show that the crab *Chasmagnathus granulatus* can be trained by means of a technique similar to the standard inhibitory avoidance method widely used in vertebrates. The salient advantages attributed to this technique may be examined in the light of the present results.

The Response is Quickly Learned

A single training trial is enough to establish an association between LC and shock.

No Pretraining is Necessary

Unlike experiments in which a pretraining or a previous shaping is necessary, this learning task does not require any previous treatment.

Memory is Stable for a Substantial Time After Training

The purpose of this work was to test a suitable training technique in order to study the effect of opiates on memory. The 3-hr memory retention might be considered "a substantial time," because such period seems to be long enough to evaluate posttraining treatment effects in the time-domain.

However, it is necessary to point out that some aspects of the training procedure are quite different from those used in passive avoidance learning with vertebrates. First, crabs are trained to avoid a light compartment instead of a dark compartment, because pilot experiments demonstrated they move very slowly from LC to DC. Second, crabs are confined to the punished side of the apparatus during the 1-min training trial rather than being allowed to escape. The purpose of this latter change in the vertebrate technique is to ensure animals stay the same interval in LC and receive the same temporal exposure to shock; because although some crabs respond with an escape reaction, others tend to remain in LC displaying a frozen defensive response [14]. Third, crabs are confined in the nonpunished side of the apparatus during the intertrial interval, because memory is disrupted by removal from the training unit. Fourth, unlike experiments with vertebrates, crabs are here adapted to the apparatus for 1 hr before training (adaptation interval), because immediately after being placed in DC they show a phase of hyperactivity, running around and attempting to climb the chamber walls.

Results indicate that there is memory retention up to 3 hr but none can be detected after 24 hr. This finding warrants further investigation aimed at testing retention over periods greater than 3 and less than 24 hr, so that a time course of memory decline could be plotted.

ACKNOWLEDGEMENTS

This work was supported by the Consejo Nacional de Investigaciones Cientificas y Tecnicas de Argentina, grants 0318/86 and PID 3-042100-85. The authors wish to thank Ms. Juliana Grichener and Mr. Angel Vidal for technical assistance.

REFERENCES

1. Balderrama, N. One trial learning in the american cockroach, *Periplaneta americana. J Insect Physiol* **26:** 499–504, 1980.
2. Brunner, D. and H. Maldonado. Habituation in the crab *Chasmagnathus granulatus*. Effect of morphine and naloxone. *J Comp Physiol* **162:** 687–694, 1988.
3. Cheney, C. D., A. Klein and R. L. Snyder. Transfer of learned dark aversion between cockroaches. *J Biol Psychol* **16:** 16–20, 1974.
4. Davenport, A. P. and P. D. Evans. Sex-related differences in the concentration of Met-enkephalin-like immunoreactivity in the nervous system of an insect, *Schistocerca gregaria*, revealed by radioimmunoassay. *Brain Res* **383:** 319–322, 1986.
5. El-Sahly, M., R. Abou-El-Ela, S. Falkmer, L. Grimelius and E. Wilander. Immunohistochemical evidence of gastero-pancreatic peptides of vertebrate types in the nervous system of the larva of a dipteran insect, the house-fly, *Ristalis aeneus*. *Regul Pept* **1:** 187–204, 1980.
6. Fanselow, M. S. and R. A. Sigmundi. Species-specific danger signals, endogenous opioid analgesia, and defensive behavior. *J Exp Psychol [Anim Behav]* **12:** 301–309, 1986.
7. Gold, P. E. The use of avoidance training in studies of modulation of memory storage. *Behav Neural Biol* **46:** 87–98, 1986.
8. Hansen, B. L., G. N. Hansen and B. Scharrer. Immunoreactive material resembling vertebrate neuropeptides in the corpus cardiacum and corpus allatum of the insect *Leucophaea maderae*. *Cell Tissue Res* **225:** 319–329, 1982.
9. Heise, G. A. Learning and memory facilitators. Experimental definition and current status. *Trends Pharmacol Sci* **2:** 158–160, 1981.
10. Hentschel, E. and H. Penzlin. Beeinflussung des Putzverhaltens bei *Periplaneta americana (L)* durch Wundsetzung, Naloxon-, Morphin- und Met-Enkephalingaben. *Zool Jb Physiol* **86:** 361–370, 1982.
11. Jarvic, M. E. and W. B. Essman. A simple one-trial learning situation for mice. *Psychol Rep* **6:** 290, 1960.
12. London, J. A. and R. Gillette. Mechanism for food avoidance learning in the central pattern generator of feeding behavior of *Pleurobranchaea californica*. *Proc Natl Acad Sci USA* **83:** 4058–4062, 1986.
13. Lovell, K. L. and E. M. Eisenstein. Dark avoidance learning and memory disruption by Carbon dioxide in cockroaches. *Physiol Behav* **10:** 835–840, 1973.
14. Lozada, M., A. Romano and H. Maldonado. Effect of morphine and naloxone on a defensive response of the crab, *Chasmagnathus granulatus*. *Pharmacol Biochem Behav* **30:** in press, 1988.
15. Maldonado, H. and A. Miralto. Effect of morphine and naloxone on a defensive response of the mantis shrimp *(Squilla mantis)*. *J Comp Physiol* **147:** 455–459, 1982.
16. Menzel, R., J. Erber and T. Mashur. Learning and memory in the honeybee. In: *Experimental Analysis of Insect Behaviour*, edited by L. Barton-Brown. Berlin: Springer, 1974, pp. 195–217.
17. Minami, H. and K. M. Dallenbach. The effect of activity upon learning and retention in the cockroach *Periplaneta americana*. *Am J Psychol* **59:** 1–58, 1946.
18. Nuñez, J. A. Sammelbienen markieren versiegten Futterquelen durch Duft. *Naturwissenchaften* **54:** 322, 1967.
19. Nuñez, J. A., H. Maldonado, A. Miralto and N. Balderrama. The stinging response of the honey bee. Effects of morphine, naloxone and some opioid peptides. *Pharmacol Biochem Behav* **19:** 921–924, 1983.
20. Pages, M., F. Jimenez, A. Ferrus, G. Ramirez and E. Gelpi. Enkephalin-like immunoreactivity in *Drosophila melanogaster*. *Neuropeptides* **4:** 87–98, 1983.
21. Remy, C. and M. P. Dubois. Immunohistological evidence of Methionine enkephalin-like material in the brain of the migratory locust. *Cell Tissue Res* **218:** 271–278, 1981.
22. Sahgal, A. A critique of the vasopresin-memory hypothesis. *Psychopharmacology (Berlin)* **83:** 215–228, 1984.
23. Sahley, C., A. Gelperin and J. W. Rudy. One-trial associative learning modifies food odor preferences of a terrestrial mollusc. *Proc Natl Acad Sci USA* **78:** 640–642, 1981.
24. Szymansky, J. Modification of innate behavior of cockroaches. *J Anim Behav* **2:** 81–95, 1912.
25. Turner, C. H. An experimental investigation of an apparent reversal of responses to light of the roach, *Periplaneta orientalis L. Biol Bull* **23:** 371–386, 1912.
26. Valenta, J. G. and M. K. Rigby. Discrimination of the odor of stressed rats. *Science* **161:** 599–601, 1968.
27. Verhaert, P. and A. De Loof. Immunocytochemical localization of Methionine-enkephalin-resembling neuropeptide in the central nervous system of the American cockroach, *Periplaneta americana*. *J Comp Neurol* **239:** 54–61, 1985.
28. Zabala, N. A., A. Miralto, H. Maldonado, J. A. Nuñez, K. Jaffe and L. de C. Calderon. Opiate receptor in praying mantis: Effect of morphine and naloxone. *Pharmacol Biochem Behav* **20:** 683–687, 1984.