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Arginine and Memory Consolidation in Praying Mantis

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Summary. A praying mantis (*Stagmatoptera biocellata*) shown a bird (*Padda oryzivora*) displays a frightening response called *deimatic reaction* (DR). Habituation of this response takes place after repeated presentation of the bird (no-DR training). Anoxia with nitrogen impairs no-DR retention but after 5 days of training the no-DR memory becomes completely consolidated.

Paper high voltage electrophoresis of a gel filtration of brain extracts revealed three distinctive cationic spots (+1, +2 and +3) and three anionic ones frequently fused to form a single spot (–complex). Changes in density of these spots during no-DR training were observed, but only increase in density at +3 proved to be clearly correlated with memory consolidation (Maldonado 1980).

- a. Biochemical analysis reported in the present paper indicate that spot +3 is a mixture of arginine and lysine (4:1).
- b. Five different amino acids, arginine, ornithine, alanine, histidine and lysine were administered immediately before the first trial of the first no-DR training session (S_1), and anoxia was given for 3 min immediately after the last trial of S_1 . The level of DRs was measured the following day (second session of no-DR training, S_2) and only arginine completely abolished the amnesic effect of anoxia (Fig. 2).

From results a) and b) it is suggested that arginine could be involved in no-DR memory consolidation.

Introduction

A praying mantis (*Stagmatoptera biocellata*) faced with a bird displays a 'frightening reaction' called

Abbreviations: DR deimatic reaction; S_1 first training session; S_2 second training session

a deimatic reaction, DR (Maldonado 1970). A full deimatic reaction which occurred following a rest interval of 6 days included the following components: a) the antennae are directed backwards, b) the mouth parts are widely opened showing the colored mandibles, c) the prothorax is raised and the forelegs are extended laterally showing a black spot on the femurs, d) the wings are elevated showing two eye-like stigma, e) the abdomen is twisted and tilted, f) a rustling noise is made by the animals' rubbing its abdomen against its wings, and g) the entire insect sways from side to side. Habituation of this response, due to repeated presentation of different species of birds has been studied (no-DR Learning, Balderrama and Maldonado 1971; Maldonado et al. 1979). They reported that when mantises are shown Java sparrows (*Padda oryzivora*) 30 or 15 times a day, a clear-cut decrease in the number and intensity of DRs is seen during 2 days of training and good retention. The waning of the DRs was demonstrated to fulfill the parametric characteristics of habituation (Thompson and Spencer 1966).

Maldonado et al. (1979) demonstrated that anoxia with nitrogen impairs no-DR retention and that this amnesic effect diminishes with training. Thus after 5 days of training the no-DR memory becomes completely consolidated. Maldonado (1980) designed experiments aimed at testing a possible correlation between the no-DR memory consolidation and changes in the levels of components of brain extracts (i.e. amino acids or peptides of low molecular weight). Paper high voltage electrophoresis of a gel filtration of brain extracts revealed three distinctive cationic spots (+1, +2 and +3) and three anionic ones frequently fused to form a single spot (–complex). Changes in density of these spots during training were observed, but only increase in density at +3 proved to be clearly correlated with memory consolidation. Similar results were obtained using a different training

procedure with the same animals (Jaffé and Maldonado 1979; Jaffé 1980). Thus it was thought appropriate to study in more detail the possible chemical correlate of these learning processes.

Experiments reported in the present paper were designed to (1) identify the components of brain extracts present in spot +3; (2) assay the influence of these components in the no-DR memory consolidation.

Materials and Methods

Animals. The animals used were adult *Stagmatoptera biocellata* which had reached maturity 20–30 days before the beginning of the experiment. All animals had been reared in individual cages at a constant temperature of 29 °C during the day and 24 °C at night with a relative humidity of 65% and 12 h of light per day. They were fed *Sarcophaga* flies every 4 days. The birds were *Padda oryzivora*.

Identification of Components Isolated by Electrophoresis

Apparatus and Training Procedure. Sixty female and sixty male mantises were trained according to the 'discontinuous procedure' described by Maldonado (1980). Mantises were placed in individual cages (14 × 10 × 10 cm) with three gauze net walls; the front wall was made of transparent celluloid. These cages were put into a compartment (20 × 20 × 30 cm) facing an opaque sliding screen. A bird was placed in another cage with a front wall of transparent Lucite. Each bird cage faced the sliding screen of the mantis compartment. Both compartments were illuminated with fluorescent tubes. During each trial the opaque sliding screen was lifted. One training session (S_n) consisted of 30 trials per day, a trial lasting 2 min with intertrial intervals of 10 min. The interval between sessions was 1 day. Mantises received 5 days of training, i.e. a period of training after which no-DR memory proves to be insensitive to anoxia with nitrogen (Maldonado et al. 1979; Maldonado 1980).

Preparation of the Brain 'Light Fraction' from Trained Animals. Immediately after the last trial of S_5 , mantises were killed by dropping them in a CO₂-acetone mixture. The brains were dissected according to the method of Maldonado et al. (1976). Groups of 20 brains from female or male mantises were pooled and homogenized in 1 ml of distilled water in a glass homogenizer equipped with a motor driven Teflon pestle. The homogenate was centrifuged at 80,000 g for 1 h and the supernatant was gel-filtered on a column of Sephadex G-75 (1.6 × 32 cm). Two fractions of material absorbing at 280 nm were eluted with distilled water as described by Maldonado et al. (1979). The more retarded fraction ('light fraction') was lyophilized and used in all subsequent experiments.

High Voltage Electrophoresis and Amino Acid Analysis. High voltage electrophoresis was performed with a Gilson Model D apparatus on Whatman N.1 paper in pyridinium acetate buffer at pH 6.5. Amino acids were detected with a cadmium-ninhydrin reagent.

Amino acid analyses were performed on a LKB 4400 automatic amino acid analyzer.

Amino Acid Treatment of Animals

Apparatus. The prothorax of each mantis was fixed to a plaster block PB (Fig. 1) by means of adhesive tape. Mantis plus block were fitted into a stand (S) by means of a supporting pin (SP). A balance (B) was used to compensate the weight of the animal. A Teflon tube (T) 0.3 mm in diameter and 5 mm long was impaled

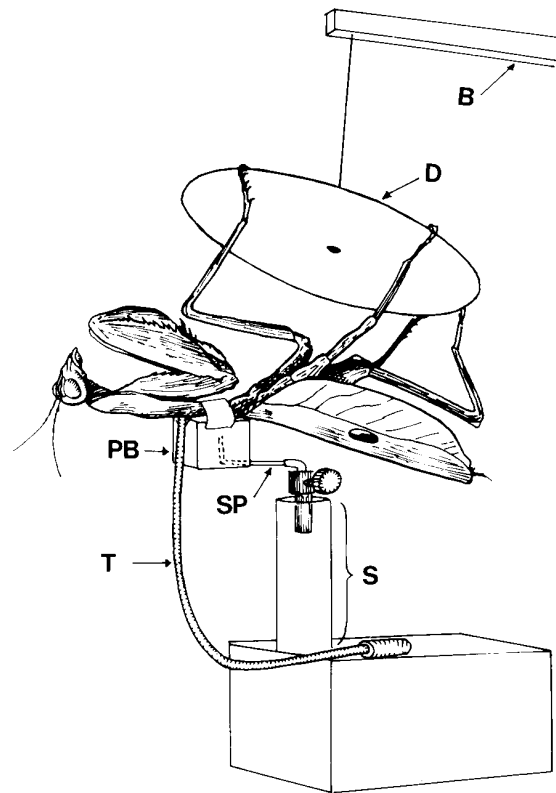


Fig. 1. The mantis holder. PB plaster block; SP supporting pin; S stand; T Teflon tube; B balance with a dish (D) to compensate the weight of the animal

into the middle dorsal prothorax. A 50 µl Hamilton syringe connected to the free end of the Teflon tube was used for injections.

Every mantis in its holder was put into a compartment (20 × 20 × 30 cm) facing an opaque sliding screen. A bird was placed in a cage with a front wall of transparent Lucite. Each bird cage faced the sliding screen of the mantis compartment and the bird cage was illuminated by fluorescent tubes. The set-up could accommodate 48 mantises and 48 birds at the same time. During each trial the opaque sliding screens were raised.

Training Procedure. Each animal received two training sessions (S_1 and S_2). Each training session consisted of 15 trials, each trial lasting 2 min with intertrial intervals of 10 min. The interval between S_1 and S_2 was 1 day. DRs were classified into five ranks, i.e. complete reaction (C): when a mantis displayed the DR with all its components (Maldonado 1970) during the entire trial; incomplete reaction (I): when the reaction lasted throughout the trial but some of the components were absent; minus complete reaction (–C): when the C was not sustained for the full 2 min; minus incomplete reaction (–I): when the I was not sustained; and no-DR when the mantis did not display any component of the DR. In order to assess the performance quantitatively, a value was assigned to each of these five categories. Four points for C, 3 for –C, 2 for I, 1 for –I and 0 points for no-DR.

Anoxia. The mantis was removed from its compartment and placed in a container through which a current of nitrogen was passed for a period of 3 min. After 40–45 s in the container the mantis became inert and its aortic pulse stopped. When the animal was put back in its cage a slow recovery began after 5–6 min. It has been suggested that the effects of nitrogen exposure, including

Table 1. Means (\pm s.e.) of DR records corresponding to the first session of training (S_1)

Group	Mean (\pm s.e.)
Control 1 (H_2O , without anoxia)	35.9 (\pm 12.0)
Control 2 (H_2O) + anoxia)	31.5 (\pm 15.8)
Arginine + anoxia	32.6 (\pm 16.7)
Ornithine + anoxia	32.1 (\pm 11.1)
Alanine + anoxia	31.9 (\pm 12.9)
Histidine + anoxia	32.7 (\pm 12.9)
Lysine + anoxia	33.2 (\pm 14.4)
Pre-24 h arginine + anoxia	33.3 (\pm 15.2)

memory disruption, are due to anoxia (Maldonado et al. 1979; Lovell and Eisenstein 1973).

Experimental Design. 160 female mantises were randomly distributed in eight groups of 20 each. *Control group 1* (H_2O , no anoxia): immediately before the first trial of S_1 the animals received an injection of 50 μ l of distilled H_2O . *Control group 2* (H_2O + anoxia): the animals were treated as above, but received anoxia immediately after the last trial of S_1 . *Arginine + anoxia group*: the animals received an injection of 50 μ l 0.8 M L-arginine. HCl (SIGMA) instead of H_2O , and anoxia immediately after the last trial of S_1 . *Ornithine + anoxia group*: the animals received an injection of 50 μ l 0.8 M L-ornithine. HCl (Eastman) and anoxia immediately after the last trial of S_1 . *Alanine + anoxia group*: an injection of 50 μ l 0.8 M L-alanine. HCl (SIGMA); anoxia immediately after the last trial of S_1 . *Histidine + anoxia group*: an injection of 50 μ l 0.8 M L-histidine. HCl (SIGMA); anoxia immediately after the last trial of S_1 . *Lysine + anoxia group*: an injection of 50 μ l 0.8 M L-lysine. HCl (SIGMA); anoxia immediately after the last trial of S_1 . *Pre-24 h Arginine + anoxia*: 24 h before the first trial of S_1 , the animals were injected with 50 μ l of 0.8 M L-arginine. HCl; anoxia was given immediately after the last trial.

Results

Identification of the Cationic Components of the 'Light Fraction' from Brain Extracts of Trained Mantises

The cationic components of the gel filtration 'light fraction' from brain extracts of trained mantises had

been previously analyzed by paper electrophoresis for 30 min at 2,400 V (Maldonado 1980). Three components, increasingly cationic, had been separated: spots +1, +2 and +3. A higher resolution of these components was achieved by increasing the voltage to 3,000 V and the duration of electrophoresis to 45 min. Under these conditions the most cationic spot (+3) was found to resolve into two components (+3a and +3b). It was also found that the electrophoretic mobilities of these components were identical with those of authentic lysine and arginine, which were added as markers. Spot +2 was identified with histidine.

These results were confirmed when the regions corresponding to these components were eluted with 20% acetic acid from an unstained electrophoretogram. The eluates were dried and then analyzed with an amino acid autoanalyzer. Spots +3a and +3b contained lysine and, respectively, arginine. No other amino acids were found.

The 'light fraction' was then analyzed and found to contain arginine and lysine in a ratio of 4:1. No differences were found in the arginine:lysine ratio in brains from male and female mantises.

The Arginine Effect on Memory Consolidation in Mantis

Results of S_1 are presented in Table 1. An analysis of variance shows that estimated variance between means is due to chance ($F=0.18$), i.e. neither the type of amino acid in the solution nor the fact that in one group the injection was administered 24 h before the first trial proved to be a significant source of variation between groups.

Figure 2 summarizes the percentage of response inhibition for each group, i.e. the difference of performance (S_2-S_1) expressed as percentage. Performance of *control group 1* (H_2O no-anoxia) rates 61.7% and

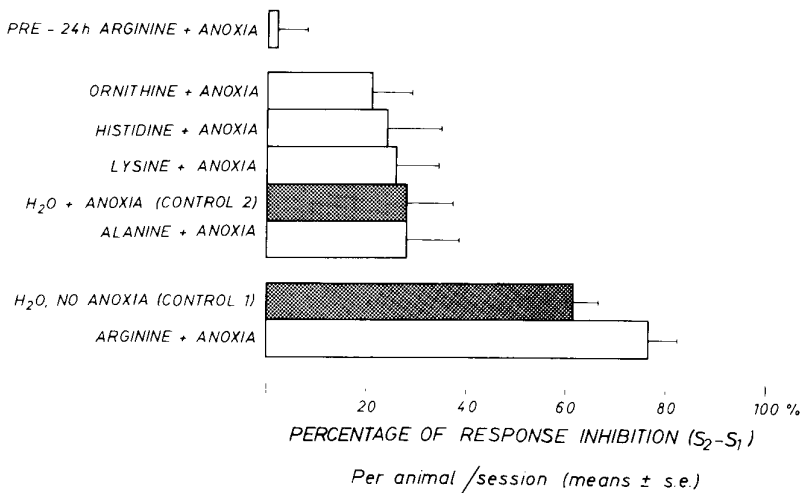


Fig. 2. Percentage of response inhibition (S_2-S_1) for the different groups. S_1 first session of training; S_2 second session of training

that of *control group 2* (H_2O +anoxia) 28.5% ($D: +33.2$; $t=3.1$; $P<0.005$), i.e. anoxia with nitrogen impairs retention on the following day.

Mantises that received injections of ornithine, histidine, lysine or alanine immediately before the first trial of S_1 and anoxia after the last trial, show a percentage of response inhibition ($S_2 - S_1$) similar to that of the *control group 2* (H_2O +anoxia) (t -test, double tailed, $\alpha=0.05$); i.e. these amino acids did not modify the amnesic effect of anoxia.

When instead arginine was administered immediately before the first trial of S_1 , the amnesic effect of anoxia was abolished. *Arginine+anoxia group* shows a percentage of response inhibition of 76.7% against 28.5% of the *control group 2* ($D: +48.2$; $t=4.4$, $P<0.001$), and against 61.7% of *control group 1* (H_2O , no anoxia) ($D: +15.0$, $t=1.7$ NS).

When arginine is given 24 h before the first trial of S_1 , the amnesic effect of anoxia seems to be enhanced (difference between the percentage of the *pre-24 h arginine+anoxia group* and that of the *control group 2* = -26.4 ; $t=2.4$, $P=0.02$).

Discussion

Maldonado (1980) showed a clear correlation between density of spot +3 in electrophoretograms obtained from brain extracts and no-DR memory consolidation. The results reported in the present paper indicate that spot +3 is a mixture of arginine and lysine (4:1), suggesting that both amino acids or one of them could be involved in memory fixation.

Animals submitted to anoxia with nitrogen immediately after the last trial of S_1 (*control group 2*) show a clearcut loss of retention in S_2 (loss of 53.8% in comparison with retention in mantises without anoxia, i.e. *control group 1*). This result is consistent with previous ones (Maldonado et al. 1979; Maldonado 1980).

Arginine administered immediately before S_1 (*arginine+anoxia group*) completely abolishes the amnesic effect of anoxia. This result strongly suggests that arginine helps memory consolidation, at least for 24 h. As arginine given 24 h before S_1 does not decrease performance during the first session (Table 1), an alternative explanation based on a depressive effect can be excluded.

Lysine, although also present in spot +3 of the electrophoretograms, is completely unable to cancel the amnesic effect of anoxia.

Also histidine, identified with spot +2 of the electrophoretograms, did not abolish the amnesic effect of anoxia. This is in line with the results of Maldonado (1980), who showed that the increase in density at position +2 was correlated with an increase of

activity during training, but not with memory fixation.

Ornithine has been discarded as precursor of arginine in insects since it cannot replace this amino acid in the diet of several species (Davis 1962; House 1958; Goldberg and De Meillon 1948; De Groot 1953; Hinton 1956). However, it has been postulated that it can be produced from arginine 'for some as yet unknown purpose' (Kilby 1963). In the present paper ornithine was tested and shown to be ineffective in eliminating the amnesic effect of anoxia.

Also alanine was found to be ineffective in canceling the amnesic effect of anoxia.

Thus, only arginine of the five amino acids tested appears to play a consolidating role on the no-DR memory.

The performance of the *pre-24 h arginine+anoxia group* suggests that a certain temporal overlapping of a high level of arginine and training is necessary in order to produce the anti-amnesic effect. The precise limits of this temporal overlapping remains a subject to be investigated. In addition, results with this group raise a rather puzzling question, since arginine given 24 h before S_1 not only has no anti-amnesic effect, but it appears to enhance the amnesic effect of anoxia, i.e. the response inhibition (S_2-S_1) of the *pre-24 h arginine+anoxia group* is significantly lower than that of the *control group 2* (H_2O +anoxia).

The conclusions reached on the basis of the results as reported are thus the following:

(1) Level of no-DR memory fixation and level of arginine in the brain increase during training. Full consolidation and a maximum level of brain arginine is reached after five days of training.

(2) Consolidation, however, is reached after a single day of training if an injection of arginine is given immediately before the first trial. An 'artificial' increase of the arginine level accelerates the process of memory consolidation.

(3) Conclusions (1) and (2) suggest that arginine is involved in memory consolidation. The way this amino acid plays its role, the persistence of its effect and other implications have yet to be investigated.

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