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European Journal of Pharmacology 298 (1996) 217–225

ejp

# Influence of a dietary α-linolenic acid deficiency on learning in the Morris water maze and on the effects of morphine

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Received 3 August 1995; revised 27 November 1995; accepted 28 November 1995

**Abstract**

Female OF1 mice were fed on a diet deficient in α-linolenic acid or on a control diet 3 weeks before mating and throughout pregnancy and lactation. Pups fed on the same diet as their mothers were used for experiments. The effects of dietary α-linolenic acid deficiency were studied in a model of learning, the Morris water maze, and on the following effects of morphine: increase in locomotor activity, modifications of rectal temperature and analgesia. In the place and in the cue versions of the Morris water maze, learning occurred at the same speed in the two diet groups; however, in the place version of the test, the level of the performance was significantly lower in the deficient mice. The probe trial and the extinction procedure did not show any difference between the two diet groups. The morphine-induced increase in locomotor activity occurred significantly earlier and was greater in the deficient diet group. Morphine induced an early hypothermia followed by a late hyperthermia; the hypothermia was significantly greater and the hyperthermia significantly smaller in the deficient mice. The pain thresholds and the morphine-induced analgesia were unmodified by the dietary deficiency. The plasma levels of morphine were similar in the two diet groups.

*Keywords:* α-Linolenic acid deficiency, dietary; Morris water maze; Learning; Morphine effect; (Mouse)

## 1. Introduction

Long-chain polyunsaturated fatty acids are components of membrane phospholipids (mainly arachidonic acid and cervonic acid). They are synthesized by successive desaturation and elongation of the precursors linoleic acid [18:2(*n*–6)] and α-linolenic acid [18:3(*n*–3)]. The precursors themselves cannot be synthesized by animals but only by plants; so, they must be obtained from diet and are said to be essential fatty acids.

Since polyunsaturated fatty acids are structural components of membrane phospholipids, they contribute to the properties of these membranes (fluidity, enzymatic activity, binding between receptors and endogenous molecules or drugs). Therefore a diet deficient in essential fatty acids is expected to induce pathological symptoms and indeed reproductive failure. In young animals, diminished growth has been observed (Burr and Burr, 1929, 1930).

More recently, the effect of a specific deficiency in fatty acids derived from α-linolenic acid has been studied and an alteration in retinal function in rats, rhesus monkeys and human infants has been observed (Neuringer et al., 1988). Behavioral studies have shown that a dietary deficiency in fatty acids derived from α-linolenic acid alters performance in some learning tasks: active avoidance in rats (Bourre et al., 1989), spatial learning in the Morris water maze in mice (Nakashima et al., 1993), brightness-discrimination learning ability and extinction of this learning in rats (Yamamoto et al., 1988). However, discordant results have also been reported, namely that this deficiency does not alter the performance in the Morris water maze (Wainwright et al., 1994).

Regarding the effects of drugs, Nakashima et al. (1993) found that mice fed on a diet deficient in fatty acids derived from α-linolenic acid showed a greater sensitivity to pentobarbital but a smaller scopolamine-induced increase in locomotor activity, and Bourre et al. (1989) observed higher mortality in response to an intraperitoneal injection of a neurotoxin, triethyltin, in rats fed on a low α-linolenic acid diet than in control rats. Since a diet deficient in α-linolenic acid alters the fatty acid profile of

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*SSDI* 0014-2999(95)00825-X

Table 1
Diet composition

| | Diet (g/kg diet) | |
|---|---|---|
| | Control | (*n*–3) deficient |
| Casein | 220 | 220 |
| DL-Methionine | 1.6 | 1.6 |
| Cornstarch | 432.4 | 432.4 |
| Saccharose | 216 | 216 |
| Cellulose | 20 | 20 |
| Mineral mixture [a] | 40 | 40 |
| Vitamin mixture [b] | 10 | 10 |
| Peanut oil [c] | 23.6 | 60 |
| Rapeseed oil [c] | 36.4 | – |

[a] Composition of mineral mixture (g/kg diet): $CaHPO_4 \cdot 2H_2O$, 15.2; $K_2HPO_4$, 9.6; $CaCO_3$, 7.2; NaCl, 2.8; MgO, 0.8; $MgSO_4 \cdot 7H_2O$, 3.6; $FeSO_4 \cdot 7H_2O$, 0.34; $ZnSO_4 \cdot H_2O$, 0.2; $MnSO_4 \cdot H_2O$, 0.2; $CuSO_4 \cdot H_2O$, 0.04; NaF, 0.03; $CrK(SO_4)_2 \cdot H_2O$, 0.02; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, $0.8 \times 10^{-3}$; KI, $1.6 \times 10^{-3}$; $CoCO_3$, $0.8 \times 10^{-3}$; $Na_2SeO_3 \cdot 5H_2O$, $0.8 \times 10^{-3}$. [b] Composition of vitamin supplement, triturated in dextrose (mg/kg diet): retinol acetate, 10; cholecalciferol, 0.0625; acetate all-*rac*-α-tocopherol, 50; menadione, 1; thiamine HCl, 10; riboflavine, 10; nicotinic acid, 45; D-calcium pantothenate, 30; pyridoxine HCl, 10; inositol, 50; D-biotin, 0.2; folic acid, 2; cyanocobalamin, 0.0135; L-ascorbic acid, 100; *p*-aminobenzoic acid, 50; choline chlorohydrate, 750. [c] Total dietary lipids: 6 g/100 g diet.

membranes in the forebrain of mice (Francès et al., 1996) and in the frontal cortex, striatum and cerebellum of rats (Delion et al., 1994), it can be expected that the properties of the receptors in these membranes are altered and consequently the effect of drugs acting on these receptors may be modified.

The aim of the present experiments was to examine, under our experimental conditions, the influence of a diet deficient in α-linolenic acid on learning performance in the Morris water maze and on various effects of morphine (modification of rectal temperature, increase in locomotor activity and analgesia). Morphine was chosen because it is a marker which is routinely used in this laboratory and because its effects in behavioral experiments are studied in this laboratory.

## 2. Materials and methods

### 2.1. Animals, diet, drug

Female OF1 mice originating from IFFA-CREDO (L'Arbresle, France) and bred in our laboratory were divided into two groups 3 weeks before mating. The two groups were fed on purified diets that were similar except for the lipids (Table 1). The total amount of lipids was 6% in each diet. In the group fed on a diet deficient in α-linolenic acid the lipid was peanut oil, and the diet contained 1200 mg of linoleic acid [18:2(*n*–6)]/100 g diet, and traces of α-linolenic acid/100 g diet. In the group fed on a control diet, the lipids were a mixture of peanut oil and rapeseed oil, and the diet contained 1200 mg of linoleic acid/100 g diet, and 200 mg of α-linolenic acid/100 g diet. Diets were given ad libitum. The quality of the oils used was carefully monitored with regard to fatty acids and antioxidants. The toxicological analysis (performed by the Institut National de la Recherche Agronomique, Dijon, France) showed the lack of any contaminants and a non-detectable level of oxidized fatty acids or of *trans* structures. The diets were prepared in the Institut National de la Recherche Agronomique (INRA-CNRZ), Jouy-en-Josas, France.

Twenty litters from 25 females and 20 litters from 23 females were obtained in the control and the deficient diet groups, respectively. At day 10 after birth, the litters were culled to keep only 10 pups maximum per dam and to preserve as many males as females as possible in each litter. At weaning (day 21), pups were stamped with a solution of picric acid, one mark corresponding to one mother; the pups were housed (seven per cage: 30 × 20 × 10 cm) without brothers or sisters in the same cage.

Morphine sulfate (Francopia, France) was dissolved in demineralized water and administered in a volume of 0.2 ml/20 g body weight. Control mice received demineralized water.

### 2.2. Morris water maze

The grey plastic tank was 80 cm in diameter and 30 cm high. A white circular platform (6 cm in diameter) was placed on a pedestal 19 cm above the floor of the tank. The tank was filled with water (22°C) to a level of 20 cm. The submerged platform was made non-visible by adding a white opacifier, LYTRON 631 (Morton International, distributed by Brenntag France, Sartrouville, France), and mixing thoroughly. Ten female mice per diet group were used for place learning and extinction. These mice were not related. Ten other female mice per diet group were used for the cue learning protocol. They were also not related.

#### 2.2.1. Place learning

Each mouse received four trials a day for 4 consecutive days. For each trial, the mouse was placed in the water facing the pool wall at one of eight possible starting locations, which were regularly distributed around the tank. In this protocol there were visual cues throughout the room including posters on the walls, a light on a wall and the experimenter. The latency to find the hidden platform was recorded with a stopwatch. If a mouse did not find the platform after 120 s of swimming, it was gently put on it. Once the mouse located the platform (or was put down on it) it was permitted to remain on it for 30 s. At the end of the four trials the mouse was dried with paper towels and returned to a holding cage positioned 40 cm under a 60-W lamp. On the fourth day of the learning test, the platform

was withdrawn and the time the mouse swam in each of the four quadrants of the tank was recorded for 100 s.

*2.2.2. Extinction*

On the days 8, 10 and 12 the mice used in the place learning test were put in the tank for 100 s. The platform had been removed. The time spent in each of the four quadrants was recorded.

*2.2.3. Cue learning*

In this protocol, the platform was rendered visible by attaching a cue to signal its position. The cue was a plastic square (5 × 5 cm) bearing slanting black and white, 1 cm width, stripes. As in the place learning protocol, the mouse was placed in the water facing the pool wall at one of the eight possible starting locations. The mice received three successive trials a day separated by 30 s of rest on the platform. The test was performed on 3 consecutive days. The latency to reach the platform was recorded with a stopwatch. The mice were dried at the end of each assay in the same way as described for place learning.

*2.3. Morphine studies*

*2.3.1. Locomotor activity*

Fifty-six male mice were used for this experiment: 28 fed on the control diet and 28 fed on the deficient diet. The animals were brought to the experimental room and half the mice in each diet group received morphine 100 mg base/kg i.p. and the other half received demineralized water. Thirty minutes later, the mice were individually placed in a photocell actimeter (Apelex, Massy, France) and the cumulative motor activity was registered at 15, 30, 45 and 60 min.

*2.3.2. Rectal temperature*

Female mice were used for this experiment: 20 fed on the control diet and 20 fed on the deficient diet. The animals were brought to the experimental room and placed in individual cages. After 30 min of adaptation, temperature was measured rectally (at 2 cm depth) with a heat-sensitive thermistor probe. Then, in each diet group, half of the mice received morphine (20 mg base/kg i.p.) and the other half received demineralized water. The rectal temperature was measured 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h and 6 h after drug administration. Each group contained 10 mice.

The area under the time-response curve (AUC) for each animal was calculated by curve fitting and integration procedures utilizing an IBM personal computer.

Since the mice were handled 9 times until the end of the experiment, this may influence the rectal temperature. Therefore, to take into account the effect of the repetitive handling, the following procedure was used to express the results: for each control mouse, the basal temperature (time 0) was subtracted from the temperature at time $t$. Then, the mean (± S.E.M.) temperature-time curve for all control mice fed on the same diet was calculated. For each morphine-treated mouse, the basal temperature (time 0) was subtracted from the temperature at time $t$. Then, the mean temperature-time curve of corresponding control mice was substracted from this temperature-time curve obtained for each mouse. Thus we obtained an area under the curve (AUC) which was at first negative and thereafter positive. The negative AUC and the positive AUC were compared in control and deficient diet groups using Student's $t$-test (unpaired, two-sided).

*2.3.3. Morphine analgesia*

Thirty-two male mice fed on each diet were used. The pain threshold was measured by the tail-flick method. The tail-flick latency to thermal stimulation was determined prior to and at 30 min, 1.0, 1.5, 2.5 and 3.5 h after morphine injection (5 mg base/kg, s.c.). The basal tail-flick latency for animals from different diet groups ranged from 1.4 to 4.5 s. A cut-off time of 10 s was used to prevent damage to the tail. The basal response was subtracted from the response after morphine, and the area under the time-response curve was calculated for each mouse. The data were expressed as mean area under the time-response curve ± S.E.M. The kinetics of the analgesic response in the two diet groups were compared by using an analysis of variance followed by Bonferroni tests.

*2.3.4. Morphine assay*

Morphine was measured by a radioimmunoassay using antibodies raised in goats against *N*-carboxymethylnormorphine linked to bovine serum albumin. Antibodies were specific to morphine and exhibited no cross-reactivity (< 0.2%) with morphine metabolites and opiate peptides (< 0.05%), as previously described (Sandouk et al., 1991). The limit of detection of morphine was 0.1 ng/ml. The day-to-day and within-day variations ranged from 6.6 to 8.2% and 1.2 to 5.0%, respectively, at concentrations ranging from 0.1 to 50 ng/ml.

*2.4. Statistics*

For place learning and cue learning, a univariate repeated measures analysis was performed using the Huynh-Feldt probability adjustment (Morrisson, 1976). This analysis was followed by post-hoc tests of specific contrasts. When the learning performances were compared, it was hypothesized that control mice would have the best learning results, thus justifying the use of one-sided tests. All results were obtained using the SYSTAT Software (SYSTAT, Evanston, IL, USA).

For morphine-induced analgesia and modifications of rectal temperature, the area under the curves were compared using Student's $t$-tests.

For the extinction of learning, the kinetics of the effect of morphine on rectal temperature, and the kinetics of

morphine-induced analgesia, a one-way analysis of variance was followed by Bonferroni tests. Pain thresholds were compared using the Mann-Whitney test. Plasma concentrations of morphine and the effect of morphine on locomotor activity were analyzed using Student's *t*-test.

## 3. Results

### 3.1. Morris water maze

#### 3.1.1. Place learning

The results (Fig. 1A) show that the latency to find the hidden platform decreased significantly over the successive learning trials in both diet groups. This indicates that the two diet groups were able to learn where the platform was located. The statistical analysis shows a parallelism between the two diet groups regarding the evolution of the scores over the trials. Thus neither of the diet groups had a faster learning curve. It may be seen from the Fig. 1A that the scores of the deficient mice were regularly higher than those of the control mice. This difference was significant.

#### 3.1.2. Probe trial

The time spent swimming in the target quadrant during the probe trial was higher than in every other quadrant for both diet groups (data not shown); however, no significant difference in the time spent swimming in the target quadrant was seen between the control diet group (mean ± S.E.M., 41.9 ± 3.2) and the deficient diet group (40.3 ± 4.7).

#### 3.1.3. Extinction (Table 2)

The results show that the duration of swimming was significantly higher in the quadrant (Q4) from which the platform was withdrawn than in every other quadrant in both control and deficient diet groups on day 8. There was also a tendency to the shortest duration of swimming in the quadrant Q8 opposite to the quadrant Q4. But there was no difference between the two diet groups. On day 10, the duration of swimming in quadrant Q4 was still higher than in the other quadrants, but the differences were not significant except for the quadrant Q8 in the control group. On day 12, no statistical significant difference appeared between any quadrants for either diet group.

#### 3.1.4. Cue learning

The results (Fig. 1B) show that the latency to find the platform decreased significantly over the successive learning trials in both diet groups, confirming the ability of the deficient mice to learn. The evolution of the scores over the trials did not differ between the deficient and the control mice, indicating that the learning did not occur faster in one diet group than in the other. Although the scores of the deficient mice were regularly higher than those of the control mice, the difference was not significant.

### 3.2. Morphine studies

#### 3.2.1. Locomotor activity

At the dose of 100 mg base/kg, morphine increased the locomotor activity in the two diet groups. The effect was only significant at 60 min in the control group. In the deficient mice, the increase in locomotor activity was greater than in control mice and occurred significantly earlier (from 30 min) (Fig. 2A,B).

#### 3.2.2. Rectal temperature

Morphine (20 mg base/kg) induced an early decrease followed by a late increase in rectal temperature. The same phenomenon was observed in both the control and deficient diet groups. The areas under the curves obtained for hypothermia on one hand and hyperthermia on the other hand showed that in the deficient diet group the hypothermia was significantly more marked than in the control diet group. Inversely, the late hyperthermia was significantly lower in the deficient diet group than in the control diet group (Fig. 3A). A further analysis of the results, taking into account the kinetics of the effects, showed (Fig. 3B) that the hypothermia lasted longer in the deficient diet group, because the effect of morphine differed significantly between the two diets at the times 1, 2 and 3 h.

Table 2
Swimming duration (s, mean ± S.E.M.) in each quadrant

| Day | Diet | Q2 | Q4 | Q6 | Q8 | *P* |
|---|---|---|---|---|---|---|
| 8 | $(n-3)^+$ | 23.9 ± 3.8 [b] | 43.4 ± 3.5 NS | 19.4 ± 2.1 [c] | 12.9 ± 1.6 [c] | $F(7,72) = 19.539$ |
| | $(n-3)^-$ | 20.9 ± 2.6 [c] | 47.1 ± 4.1 | 19.7 ± 2.8 [c] | 11.0 ± 2.8 [c] | $P < 0.0001$ |
| 10 | $(n-3)^+$ | 22.3 ± 5.8 NS | 41.2 ± 5.1 NS | 21.3 ± 3.0 NS | 14.8 ± 2.8 [b] | $F(7,72) = 4.868$ |
| | $(n-3)^-$ | 19.5 ± 1.5 NS | 36.7 ± 5.3 | 20.1 ± 2.0 NS | 22.9 ± 5.1 NS | $P = 0.002$ |
| 12 | $(n-3)^+$ | 23.9 ± 4.7 NS | 31.3 ± 2.4 NS | 21.8 ± 2.7 NS | 22.7 ± 3.4 NS | $F(7,72) = 1.930$ |
| | $(n-3)^-$ | 25.0 ± 3.9 NS | 32.9 ± 5.2 | 17.8 ± 2.0 NS | 24.3 ± 2.9 NS | $P = 0.0771$ |

$(n-3)^+$: control diet group, $(n-3)^-$ diet group deficient in α-linolenic acid. The platform was removed from the quadrant Q4. The last of the learning sessions was performed on day 4. The one-way analyses of variance were followed by Bonferroni tests. Intra-diet significance, reported on the right of the values, is relative to data for the Q4 quadrant. Inter-diet significance is reported under a value. [b] $P < 0.01$, [c] $P < 0.001$.

### 3.2.3. Pain threshold and morphine-induced analgesia

The pain threshold did not differ between the two diet groups (Table 3). The analgesic effect of morphine, measured as the area under the curve $AUC_{0 \rightarrow 210\,min}$, did not differ significantly between the two diet groups (Table 3). To take into account a possible difference in the kinetics of morphine according to the diet, an analysis of variance followed by Bonferroni tests was performed (Fig. 4). The results show that in spite of a tendency to an early (30 and 60 min) smaller and a late (150 and 210 min) greater analgesic effect in deficient mice, the differences were never significant.

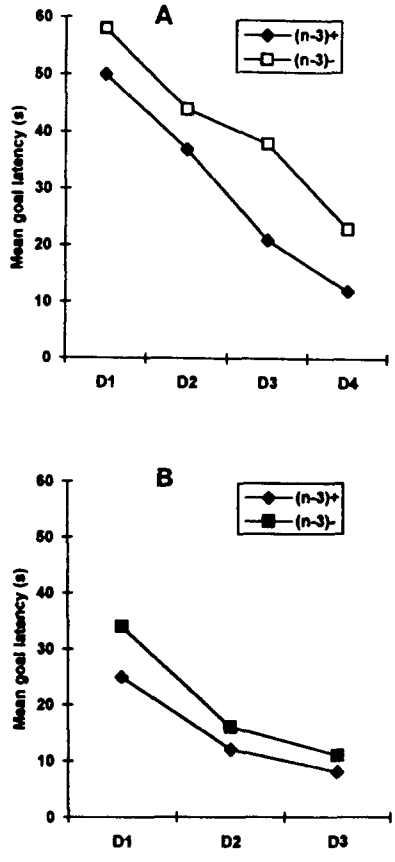


Fig. 1. Performance in the Morris water maze. (A) Latency to find the hidden platform over the 4 days ($D_1$-$D_4$) of training. The score on each day represents the average of four trials. The evolution of the scores (parallelism of the curves) is similar in the two diet groups (Huyn-Feldt $\epsilon = 0.94$, $F(3\epsilon,54\epsilon) = 0.167$; $P = 0.91$). The reduction in the latency to find the platform over the trials (learning) is significant in the two diet groups (Huyn-Feldt $\epsilon = 0.94$; $F(3\epsilon,54\epsilon) = 7.58$; $P = 0.0004$). Time latency to find the platform differed according to the diet over the trials ($F(1,18) = 3.28$; $P = 0.044$, one-sided). (B) Latency to find the labelled platform over the 3 days ($D_1$–$D_3$) of training. The score on each day represents the average of three trials. The evolution of the scores (parallelism of the curves) is similar in the two diet groups (Huyn-Feldt $\epsilon = 0.62$; $F(2\epsilon,34\epsilon) = 0.243$; $P = 0.68$). The reduction in the latency to find the platform over the trials (learning) is significant in the two diet groups (Huyn-Feld $\epsilon = 0.62$; $F(2\epsilon,34\epsilon) = 9.42$; $P = 0.004$). Time latency to find the platform did not differ according to the diet over the trials ($F1,17) = 1.11$; $P = 0.15$, one-sided).

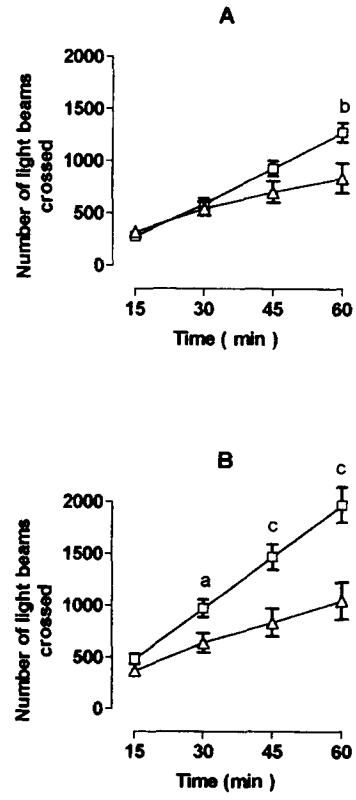


Fig. 2. (A) Control diet group. (B) Diet group deficient in $\alpha$-linolenic acid. Effect of morphine (□, 100 mg base/kg, i.p.) and demineralized water (△) on locomotor activity. Results (number of light beams crossed) are the means ± S.E.M. for 14 mice in each group. Significance is expressed vs. the corresponding control mice. Student's $t$-test: [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

### 3.2.4. Plasma levels of morphine

The plasma levels of morphine did not differ according to the diet group at any time studied, 60, 90, 120, 150 and 180 min after morphine administration (Table 4).

### 3.3. Forebrain fatty acids

In the present study, the effect of the diets on the brain fatty acid composition is not reported in detail because this composition has been studied in the same species fed on similar diets under the same conditions (Francès et al., 1996). The main alterations are described.

The method used for analysis of forebrain fatty acids has been previously described (Clément and Bourre, 1993). Analysis of the forebrain fatty acid profile (m ± S.D. of four determinations; % of total fatty acids) indicated: (1) no difference in the total saturated fatty acids (45.68 ± 0.36 for control diet; 45.74 ± 0.36 for deficient diet); (2) no difference in the total amount of monounsaturated fatty acids (19.38 ± 0.42 for control diet; 19.35 ± 0.43 for deficient diet); (3) a significant increase in the polyunsaturated fatty acids derived from linoleic acid in the deficient diet group (15.78 ± 0.19 for the control diet; 27.27 ± 0.78

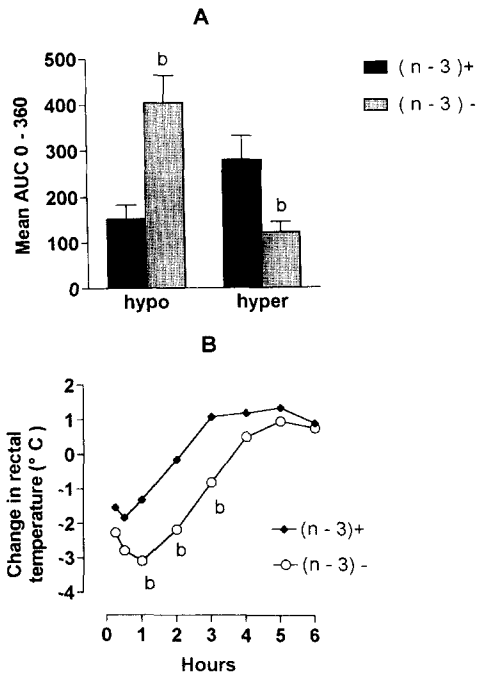


Fig. 3. Effect of morphine on rectal temperature. (A) Changes in rectal temperature [early hypothermia (hypo) and late hyperthermia (hyper)] in response to morphine (20 mg base/kg i.p.) as represented by $AUC_{0 \rightarrow 360min}$ in mice fed on either a control or a deficient diet. Ten mice were used in each group. Mann-Whitney test (unpaired, two-sided). (B) Kinetics of the effects of morphine (20 mg base/kg, i.p.) on rectal temperature in mice fed on either a control or a deficient diet. One-way analysis of variance: $F(15,144) = 23.683$, $P < 0.001$. Bonferroni tests [b] $P < 0.01$.

Table 3
Pain threshold and analgesic effect of morphine (5 mg base/kg, s.c.) in the control $(n-3)^+$ and the deficient $(n-3)^-$ diet groups

| | $(n-3)^+$ | $(n-3)^-$ | $P$ |
|---|---|---|---|
| Pain threshold (s) | 3.1 ± 0.2 (32) | 3.6 ± 0.3 (32) | 0.219 NS |
| $AUC_{0 \rightarrow 210min}$ | 719 ± 64 (32) | 792 ± 70 (32) | 0.44 NS |

(): number of values. Values are given as the means ± S.E.M. For pain threshold, the Mann-Whitney test (unpaired, two-sided) was used. For the AUC the Student's $t$-test (unpaired, two-sided) was used.

Table 4
Plasma levels of morphine in the control $(n-3)^+$ and the deficient $(n-3)^-$ diet groups

| Time (min) | $(n-3)^+$ | $(n-3)^-$ | $P$ |
|---|---|---|---|
| 60 | 308.1 ± 33.6 (4) | 394.1 ± 94.9 (5) | NS |
| 90 | 124.8 ± 19.9 (6) | 120.7 ± 25.5 (4) | NS |
| 120 | 30.2 ± 9.2 (4) | 22.3 ± 1.8 (4) | NS |
| 150 | 14.5 ± 3.8 (6) | 21.2 ± 5.4 (4) | NS |
| 180 | 26.3 ± 8.9 (4) | 15.5 ± 1.3 (5) | NS |

The mean (± S.E.M.) plasma levels of morphine are expressed in ng base/ml. Each sample was obtained from 2–3 mice. (): Number of samples. Student's $t$-tests (unpaired, two-sided).

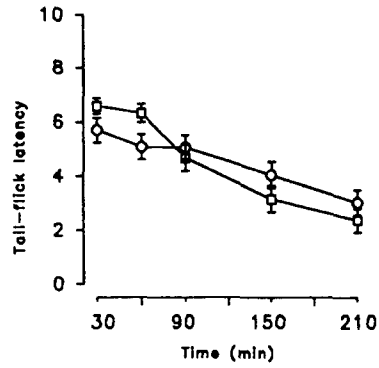


Fig. 4. Effect of morphine (5 mg base/kg, s.c.) on the tail-flick latency of mice fed on either a control (□) diet or a deficient (○) diet. One-way analysis of variance $F(9,279) = 15.2636$, $P < 0.0001$. Comparisons between the two diets for each time of measure did not indicate any significant difference, using the Bonferroni test. $n = 32$ mice in each diet group.

($P < 0.001$) for the deficient diet); (4) a significant decrease in the polyunsaturated fatty acids derived from α-linolenic acid in the deficient diet group (19.16 ± 0.20 for the control diet; 7.64 ± 0.18 ($P < 0.001$) for the deficient diet). As a whole, these data show that a dietary deficiency in α-linolenate (1/6 α-linolenic acid for deficient diet/control diet) induces a reduction in the polyunsaturated fatty acids derived from α-linolenic acid of 0.4/1 in the brain, an effect which is compensated for by an augmentation in the polyunsaturated fatty acids derived from linoleic acid of 1.73/1.

## 4. Discussion

Mice from both diet groups learned the place version of the Morris water maze because the time latency to reach the platform decreased with the number of trials. Learning was the same in both diet groups because the two curves did not differ in their parallelism. However, the goal latency was significantly higher in the deficient diet group. This result is in accordance with the results of Nakashima et al. (1993) but not with those of Wainwright et al. (1994). In the probe trial, no difference was found between the deficient and the control diet groups, as was also found by Wainwright et al. (1994). In the extinction procedure, the time spent on day 8 in the quadrant from which the platform had been withdrawn was significantly higher than the time spent in any other quadrant for both the deficient and the control mice but there was no difference between the two diet groups. This means that mice from both diet groups had an equally good memory for the platform location. On days 10 and 12, the time spent in the target quadrant decreased, and on day 12 did not differ from the time spent in any other quadrant. There was also no difference in the time spent swimming in the target quadrant between the deficient and control diet groups. This

means that extinction occurred at the same speed in the two diet groups.

In the cue version of the Morris water maze, a significant learning (decrease in the goal latency) effect occurred in both diet groups with the same speed (parallelism of the curves). But in contrast with the place version of the test, the latency to reach the platform did not differ significantly in the deficient and control diet groups in spite of a tendency to a higher goal latency in the deficient diet group. These results are in accordance with those of Wainwright et al. (1994). They indicate that motor performance and motivation did not differ between the two diet groups.

Taken together, these results show that, in the Morris water maze under our experimental conditions, a dietary deficiency in $\alpha$-linolenic acid alters the level of learning (the time to reach the goal) in the place version but not in the cue version of the test. In addition, neither the speed of learning nor its extinction nor memory was altered in the deficient mice. The increase in goal latency in the groups fed on a diet deficient in $\alpha$-linolenic acid may have several explanations. First, the swimming speed of deficient mice may be slower; however, Wainwright et al. (1994) did not find such an effect, and it is unlikely that such a reduced swimming speed is significant in the place learning protocol and not in the cue version protocol. Second, an alteration in visual function may have rendered the platform more difficult to find for the deficient group than for the control diet group (which would explain the better performance of the control mice in the place version of the test) and more difficult when the cues were spatial ones than when the platform was labelled in the pool (which would explain why the goal latency of deficient mice was significantly higher in the place version and not significantly higher in the cue version of the test). Alternatively, the processing of visual information, rather than visual function itself, may be slower in the deficient diet group than in the control diet group.

Several reports indicate that morphine exerts biphasic effects on locomotor activity, effects which depend on the dose and on the time (Dafters and Taggart, 1992). Different mice strains are very different in their sensitivity to the motor effects of morphine (Brase et al., 1977). In this study, the dose of 100 mg/kg was chosen on the basis of previous experiments conducted with the same strain in this laboratory. With this dose, the only effect obtained was an increase in locomotor activity. This activity is qualitatively so dissimilar to normal locomotor activity that it has been called 'running' (Brase et al., 1977). Morphine administration induced running in both control and deficient diet groups. However, the effect occurred significantly earlier in the deficient diet group and, for a same time of measurement, was greater. This greater effect of morphine in deficient mice does not result from a pharmacokinetic difference in plasma levels of morphine. Morphine-induced running can be decreased by the dopamine $D_1$ receptor antagonist SCH 23390 (*R*-(+)-8-chloro-2,3,4,5 tetrahydro-3-methyl-5-phenyl-1*H*-benzazepine-7-ol maleate) and the dopamine $D_2$ receptor antagonist sulpiride, as well as by the opiate receptor antagonist naloxone (Zarrindast and Zarghi, 1992). In addition, Di Chiara and Imperato (1988), in a brain dialysis study, reported that the morphine-induced stimulation of behavior, including locomotion, was associated with stimulation of dopamine release in the striatum and nucleus accumbens of the rat. Thus it can be suggested that the dopaminergic function linked to morphine-induced locomotor stimulation is more important in deficient mice.

Delion et al. (1994) observed that rats fed on a diet deficient in $\alpha$-linolenic acid had a decrease in the density of dopamine $D_2$ receptors in the frontal cortex but not in the striatum. Such a decrease may be a receptor adaptation to an increased release of dopamine. To our knowledge, no measurement of dopamine $D_2$ receptor number or of dopaminergic function has been done in the nucleus accumbens of rodents fed on a diet deficient in $\alpha$-linolenic acid. Therefore, no biochemical data are available to confirm or to refute the hypothesis of an increased dopaminergic function in the nucleus accumbens.

Alternatively, the greater effect of morphine on locomotor activity in deficient mice may result from a different reactivity of some opiate receptors. It has been shown that DTLET ([D-Thr$^2$,Leu$^5$]enkephalyl-Thr$^6$), a specific agonist of $\delta$ opiate receptors, increases motility only when injected in the nucleus accumbens whereas DAGO ([D-Ala$^2$,MePhe$^4$,Gly-ol$^5$]enkephalin), a specific agonist of $\mu$ opiate receptors, induces an early hypomotility followed by a late hypermotility when injected in the same structure (Dauge et al., 1988). So, the $\alpha$-linolenic acid deficient diet may have increased the reactivity of $\delta$ receptors and/or reduced that of $\mu$ receptors. These proposals are only working hypotheses and require further experimentation.

The biphasic effect of morphine on rectal temperature (early hypothermia and late hyperthermia) has been described a long time ago (Gunne, 1960). The dose used in the present experiments (20 mg/kg) was chosen according to the results of Glick (1975) and also on the basis of previous experiments performed with the same mouse strain in our laboratory, because it induces a clear biphasic effect. In the present experiments, the biphasic effect of morphine was observed in both control and deficient diet groups. However, in deficient mice, the hypothermia and the hyperthermia were significantly greater and smaller, respectively, than in the control diet group. From Fig. 3B it is clear that the hypothermia was more pronounced at a given time and, in addition, lasted longer in the deficient diet group. So, it is possible that the smaller hyperthermia in the deficient diet group reflects the difficulty to overcome the previous hypothermia and is not a meaningful phenomenon per se. The mechanism underlying the effect of morphine on rectal temperature is not known. The hypothermia is antagonized by naloxone (Geller et al.,

1983) and by naltrexone (Numan and Lal, 1981); the hyperthermia is also antagonized by naloxone (Geller et al., 1983; Vezina and Stewart, 1985) and naltrexone (Numan and Lal, 1981). So, these results suggest a possible alteration of opiate receptor sensitivity by a dietary deficiency in $\alpha$-linolenic acid.

The pain threshold did not differ in the two diet groups. Using another model (hot-plate), Wainwright et al. (1994) found an increase in the pain threshold in mice fed on a diet deficient in $\alpha$-linolenic acid for three generations, whereas such an increased threshold (which was strain specific) was also observed in rats fed on a diet not deficient but on the contrary enriched in $\alpha$-linolenic acid for 3–4 weeks only (Yehuda et al., 1986). Interpretation of these data is rendered difficult by the use of different experimental conditions (model, species, duration of the diet). The conditions of morphine administration (5 mg/kg, s.c.) used in the present experiments were chosen on the basis of previous results obtained in our laboratory for the same strain showing a clear analgesic effect. The analgesic effect of morphine did not differ significantly in the two diet groups in spite of a tendency to an early smaller and a late greater analgesic effect in deficient mice. These results are in agreement with the lack of a significant difference in the plasma levels of morphine in deficient and control mice.

In conclusion, the present data show that the mice fed on a diet deficient in $\alpha$-linolenic acid did not differ in an important way from the control mice regarding their performance in the Morris water maze. The only significant difference observed (learning deficiency in the place version of the Morris water maze) may reflect a slight deficiency in visual function or in information processing rather than a true impairment in learning. Regarding the effect of morphine, it was noticeable that its effect was unchanged (analgesia), increased (running activity-hypothermia) or decreased (hyperthermia). This variety of effects may reflect the diversity of the opiate receptors and pathways involved. These results suggest that more attention should be paid to the influence of diet and membrane structure on drug responses.

## Acknowledgements

This study was supported by the INSERM. We thank Dr Marcel Debray for his help in statistical analysis.

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