ELSEVIER

PII S0024-3205(96)00164-6

Life Sciences, Vol. 58, No. 21, pp. 1805-1816, 1996

0024-3205/96 $15.00 + .00

EFFECT OF DIETARY α-LINOLENIC ACID DEFICIENCY ON HABITUATION

Henriette Frances [1], Claire Monier, Michel Clement, Arlette Lecorsier, Marcel Debray and Jean-Marie Bourre

INSERM U 26 Hopital Fernand Widal, 200 rue du Fbg. St Denis, 75475 Paris Cedex10, France

(Received in final form March 20, 1996)

Summary

Three weeks before mating, two groups of SWISS OF1 mice were fed a diet that was similar but contained either peanut oil poor in alpha-linolenic acid [18 3 (n-3)] (n-3 deficient = deficient mice = $(n\text{-}3)^-$) or peanut + rapeseed oil rich in alpha-linolenic acid (n-3 nondeficient = controls = $(n\text{-}3)^+$) Pups, fed the same diet as their dams, aged 45 to 62 days were used for brain lipid analysis and for behavioral experiments, aimed at determining whether there is a relation between the dietary intake of alpha-linolenate and a simple form of learning habituation The behavior of mice was compared using four models exploration recorded in a photocell actimeter, activity in an open-field, duration of immobility in the forced swimming test and number of escape attempts from a small closed space Habituation was measured by testing the mice in the same situation after some time had elapsed since the first test Exploration in the photocell actimeter was significantly reduced between day 1 and 4 in nondeficient mice, but, not in deficient mice The number of square crossings in the open-field was significantly reduced on the second test neither in the control nor in the deficient mice In the forced swimming test, the habituation (increase in duration of immobility) was significantly greater (255%) in nondeficient than in deficient mice (163%) In the escape attempt experiment, the habituation showed a trend to be greater in controls than in deficient mice ($p=0.061$) and was significantly greater in females than in males ($p=0.028$) These results suggest that a simple form of learning, habituation, occurs more slowly in mice fed a diet deficient in alpha-linolenic acid

Key Words: α-linolenic acid deficiency, habituation, diet

The brain concentration of lipids is very high (the highest after adipose tissue) and a change in their composition could alter brain function. Alpha-linolenic acid (18 3 (n-3)) is synthesized in plant but not animal tissues it must be supplied by diet Following ingestion, α-linolenic acid is converted to long chain, highly unsaturated fatty acids One of them is docosahexaenoic acid (22 6 (n-3)) which is one of the major unsaturated fatty acids of phospholipids in several tissues including which brain It has been shown that a diet deficient in alpha-linolenic acid results in altered composition of cells, organelles and synaptic membranes in the nervous system and leads to enzymatic perturbation, electroretinogram anomalies, earlier mortality in response to an intra-

Corresponding author Dr H Frances - INSERM U 26 - Hopital Fernand Widal, 200 rue du Faubourg St Denis 75010 Paris, FRANCE Fax 40 64 40 64

peritoneal injection of a neurotoxin, triethyltin, and reduced learning ability (1,2,3,4). The aim of the present study was to assess the effect of a diet deficient in n-3 fatty acids on a simple form of learning: habituation. This choice is based on the lack of experiments relative to this form of learning. In addition, the different models used have been chosen to avoid the interference of a motivational component (such as hunger) or sensory components (such as pain or visual perturbation) as recommended recently by Wainwright (5) who underlined the pitfalls to be avoided in the interpretation of the effects on learning behavior : "when asserting cognitive differences, the onus is on the experimenter to demonstrate that the observed behavioral effects are independent of those on motivational or sensory thresholds".

Methods

Subjects : Female (Swiss OF1) mice originating from IFFA-CREDO (L'Arbresle, France) and bred in our laboratory were divided into two groups three weeks before mating. The two groups were then fed purified diets that were similar except for lipids. The total amount of lipids was 6% in each diet. In the group fed a diet deficient in α-linolenic acid [$(n-3)^-$], the lipids were peanut oil containing 1200 mg of linoleic acid [18 :2(n-6)]/100 g diet and traces of α-linolenic acid [18 : 3(n-3)]/100 g diet. In the group fed the control diet [$(n-3)^+$], the lipids were a mixture of peanut oil and rapeseed oil containing 1200 mg of linoleic acid [18:2 (n-6)] / 100 g diet ; and 200 mg of α-linolenic acid [18:3 (n-3)]/100 g diet. Diets were given *ad libitum*. The quality of the oils used was carefully examined 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 by the Institut National de la Recherche Agronomique (INRA-CNRS), 78350 Jouy-en-Josas, France. The pups from 12 dams in the $(n-3)^-$ diet group and from 9 dams in the $(n-3)^+$ diet group were fed the same diet as their dams and were used for behavioral tests from day 45 on. Breeding and experimental rooms were thermostatically maintained at 21 ± 1°C. At weaning all pups were gathered in four large cages : one cage for each of the four groups $(n-3)^-$ males, $(n-3)^-$ females, $(n-3)^+$males, $(n-3)^+$females. Then 5 to 7 pups taken at random from each group were gathered in the same cage. Experiments were performed between 10 a.m and 4 p.m by an observer blind to the diets.

Experimental design and material

Lipid analysis : Four male mice (55 days old) of each group were anesthetized with diethylether and killed by decapitation. Forebrains were quickly removed, lyophilized and stored at -70°C until the lipid analyses were performed. Heptadecanoic acid was added as internal standard and the forebrain total fatty acids were transesterified according to the method of Lepage and Roy (6). The methyl esters were analyzed on a Delsi gas chromatograph equipped with a flame ionization detector and a silica capillary column (length 25m, internal diameter 0.22 mm, stationary phase BPX 70 SGE France). Helium was used as the carrier gas. The oven temperature was programmed to maintain 165°C for 12 min, then to increase by 1.5°C/min up to 215°C; the injector and detector temperatures were maintained respectively at 230°C, and 250 °C. Peaks were identified by comparison with authentic commercial standards and with mixtures of known fatty acid composition. Areas were calculated with a Merck-Hitachi 2 500 integrator, and fatty acid concentrations were reported as percent of total fatty acid content.

Actimeter : A photocell actimeter (APELEX, 91300 Massy, France) was used. Mice were individually placed in macrolon cages (25x20x10 cm) equipped with 2 infrared photoelectric cells (in the middle of the longer and shorter sides) 2 cm above the floor. The cages were located in an

aerated cupboard without illumination other than that given by the air holes. Naive male mice, 45 days old, were used.

Experiment 1: Locomotor activity Measurements were started immediately after introduction of the animals into the actimeters and counts were automatically recorded for 20 min.

Experiment 2: Exploration, Reactivity to noise. On day 1 animals were placed in an actimeter and the exploratory activity was recorded for 5 minutes, then the mice were replaced in their maintenance cage. On day 4, these mice were again placed in the actimeter, their activity recorded for 5 min and then a noise was produced just in front of the actimeter. This noise consisted in one timer ringing 4 consecutive times. Motor activity was recorded for a further 40 min.

Open-field : Mice, 55 days old, used in this experiment had undergone the actimeter experiments 10 days earlier. A wooden white painted open-field (50x50x25 cm) in a light and sound attenuated chamber was used. The floor was divided by black lines into 25 squares (10x10 cm). An object was placed on square 2,3. A mouse placed in the middle of the open-field was observed for 5 minutes. The number of squares crossed and, rearings against the walls were counted as well as the number of explorations of the object and of defecations. The experiment was repeated on day 2 with the mice tested in the same order as on day 1.

Escape attempts : Naive male and female mice, 45 days old, were used. Mice were individually tested under a transparent beaker (height 14 cm; diameter 10 cm) inverted on a rough surface glass plate. The number of escape attempts was counted for the first 2 min of observation. An attempt at escape was defined as any one of the following: (a) the two forepaws were leant against the wall of the beaker, (b) the mouse was sniffing, its nose into the spout of the beaker, (c) the mouse was scratching the glass floor. There was no minimal duration for one attempt. For a longlasting attempt, a new attempt was counted for each period of 3 sec. The same experiment was performed 3 times at three hour intervals.

Forced swimming test : The male mice used in this experiment had undergone the escape attempts or actimeter experiments 12 days earlier ; so, they were 57 days old. On day 1, mice were individually forced to swim inside a beaker (height 18 cm, diameter 10 cm) containing 12 cm of water maintained at 23± 1°C from which they cannot escape. At the beginning of the test, the mice swim vigorously, then after nearly 2 minutes they adopt a characteristic immobile posture in they remain floating in the water making only those movements necessary to keep their heads above water. The duration of immobility was measured separately during each of the 5 minutes of the test. After 5 minutes in the water, they were removed and allowed to dry in a paperfurnished cage under a lamp before being returned to their home cage. They were replaced in the beaker 24 h later, and the same experiment was performed.

Statistical analysis of the results. For each experiment, a univariate repeated measures analysis was performed, using, for more than two repeated measures, the Huynh-Feldt probability ajustment (7). The between-group factors were diet for the actimeter, open-field and forced swimming test, and diet and sex for the escape attempts test. The within-group factors were day for the actimeter and open-field test, time for the escape attempts test, and day and time for the forced swimming test.

This analysis was followed by post-hoc one sided or two sided t-tests of specific contrasts : when comparing the scores of control and deficient mice on the first day, or time, two-sided tests were used ; when comparing the learning performances, it was hypothesized that control mice would have the best learning results, justifying onesided tests. All results were obtained using the SYSTAT Software (SYSTAT, inc., Evanston, IL, USA).

the (n-3)$^+$ group, the score was higher on the first day and did not decrease on the second day The frequency of exploration of the object did not differ between the two diet

TABLE 2
Forebrain fatty acids

Fatty acid	(n-3)$^+$ diet	(n-3)$^-$ diet
14:0	0 10 ± 0 01	0 11 ± 0 02
16 0	20 41 ± 0 42	20 70 ± 0 33
18 0	23 54 ± 0 42	23 21 ± 0 18
22 0	0 38 ± 0 02	0 40 ± 0 03
24:0	0 57 ± 0 16	0 54 ± 0 04
Σ Saturated	45 68 ± 0 36	45 74 ± 0 36
16:1n-9	0 25 ± 0 03	0 26 ± 0 02
16:1n-7	0 46 ± 0 06	0 44 ± 0 04
18:1n-9	13 57 ± 0 26	13 31 ± 0 29
18:1n-7	3 42 ± 0 16	3 64 ± 0 09
20:1n-9	1 11 ± 0 13	1 10 ± 0 06
20:1n-7	0 31 ± 0 04	0 34 ± 0 02
22:1n-9	0 16 ± 0 02	0 15 ± 0 01
22:1n-7	0 10 ± 0 01	0 11 ± 0 01
Σ MUFA	19 38 ± 0 42	19 35 ± 0 43
18:2n-6	0 34 ± 0 01	0 30 ± 0 01 **
20:3n-6	0 43 ± 0 09	0 29 ± 0 04 *
20:4n-6	11 11 ± 0 22	11 54 ± 0 23 *
22:4n-6	3 02 ± 0 18	3 89 ± 0 14 **
22:5n-6	0 87 ± 0 05	11 25 ± 0 83 ***
Σ n-6	15 78 ± 0 19	27 27 ± 0 78 ***
22:5n-3	1 62 ± 0 19	1 63 ± 0 10
22:6n-3	17 54 ± 0 26	6 01 ± 0 17 **
Σ n-3	19 16 ± 0 20	7 64 ± 0 18 **
n-6+ n-3	34 94 ± 0 33	34 91 ± 0 69
n-6/n-3	0 82 ± 0 01	3 57 ± 0 16 **

Effect of diet deficient in α-linolenic acid on the profile of fatty acids in the forebrain of 60-day-old mice Means (±S D) of 4 determinations (4 mice) are presented for each diet Results are expressed as percentages of total fatty acids.
* $p< 0.05$, ** $p< 0.01$, *** $p< 0.001$

groups either on the first or the second day. The percentage of mice defecating during the 5 min test did not differ between the groups on the first or second day, but the percentage was higher on the second day (Table 4).

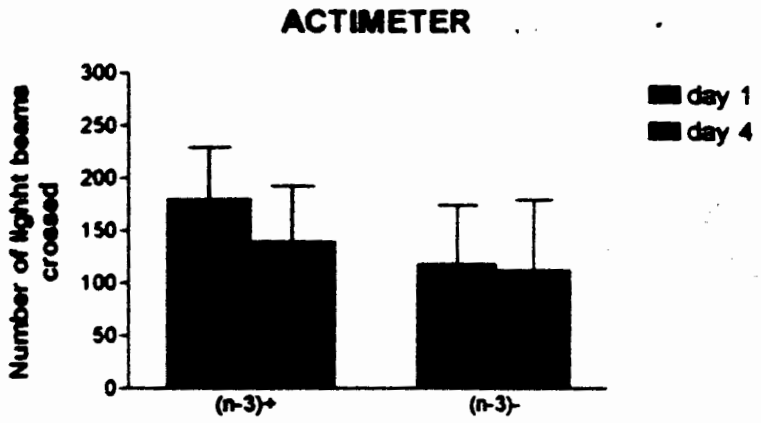

Fig. 1

The number of light beams crossed was recorded for 5 minutes. The means (± S.D.) are reported for 27 mice per group.

The difference between the scores at D4 and D1 was significantly greater in the control diet group than in the deficient diet group ($t=1.82$, $df=52$, $p=0.037$, one-sided test) The difference between the scores of the control and deficient mice on D1 was not significant.

TABLE 3

Reactivity to noise

	$(n\text{-}3)^-$	$(n\text{-}3)^+$
without noise	731.2 ± 325.6 n=12	581.3 ± 216.3 n=12
with noise	599.4 ± 370.8 n=15	592.9 ± 231.0 n=15

Note. Mean number (± S.D.) of light beams crossed in the actimeter between the 5th and the 45th min of test without and with a noise just after the 5th minute.

TABLE 4

Open-field

		Day 1	Day 2
Rearings m ± S.D.	$(n\text{-}3)^+$ $(n\text{-}3)^-$	10.8 ± 11.5 12.4 ± 13.5	8.0 ± 11.0 13.0 ± 14.0
Mice defecating %	$(n\text{-}3)^+$ $(n\text{-}3)^-$	40 40	60 72

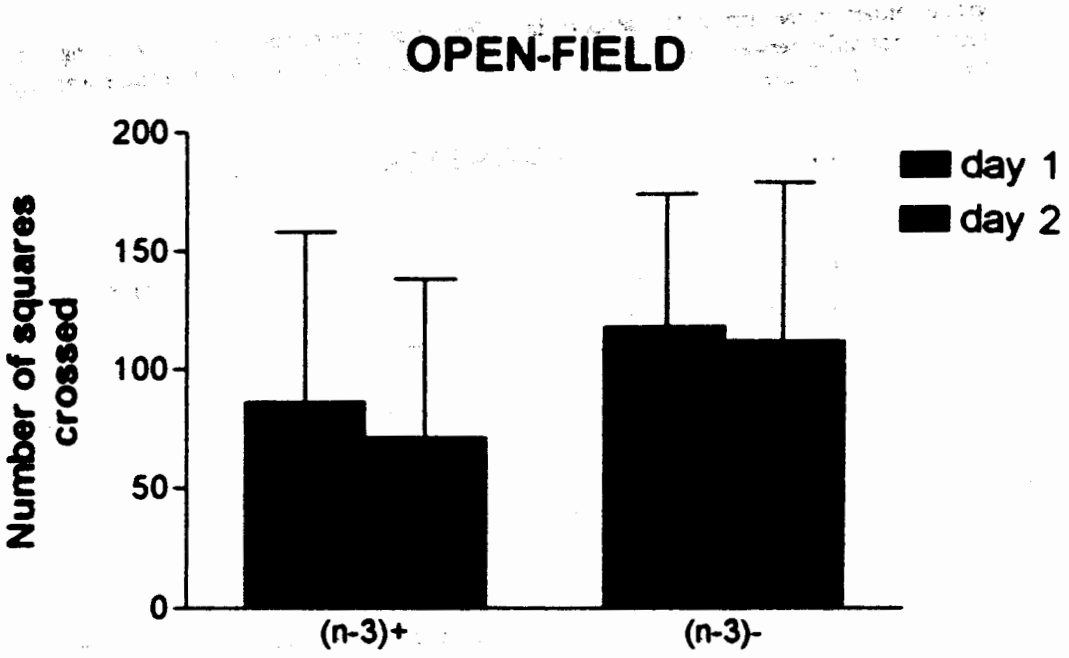

Fig. 2.

The number of squares crossed was recorded during a 5 minutes session. The means (± S D) are reported for 25 mice per group.

There was no significant interaction between diets and days. When pooling the results of D_1 and D_2, the scores of control mice was lower than that of deficient mice ($t=2.64$, $df=48$, $p=0.011$, two-sided test).

The difference in the scores at D2 and D1 did not differ significantly between controls or deficient mice.

Escape attempts: On the first test, results were as follows: number of escape attempts (m±S.E.M.), females $(n-3)^+$diet : 29.2±2.7 (n=13) , females $(n-3)^-$ diet : 21.0±2.7 (n=13) ; males $(n-3)^+$diet : 26.8±2.7 (n=13) , males $(n-3)^-$ diet : 29±3.5 (n=13). Statistical analysis of the results at time 0 showed no effect of sex or diet and no interaction. Following repeated exposure to the same environment, the number of escape attempts decreased in each group. However, the decrease was more rapid in the $(n-3)^+$ than in the $(n-3)^-$group (Fig.3 A and B) for both males and females. The number of escape attempts (% of baseline), for the 3rd test (time 6 hours) was 37% in the $(n-3)^-$diet vs 22% in the $(n-3)^+$ diet for males, and 21% in the $(n-3)^-$ diet vs 13% in the $(n-3)^+$ diet for females. The effect of the diet on the curve profile (best habituation in the $(n-3)^+$control group) was borderline significant ($p=0.06$). The decrease in the scores between time 0 and 3 hours (habituation) was significantly higher for females than for males.

Forced swimming test: The duration of immobility showed a similar increase with time for the two groups on day 1 (Fig. 4). On the second day the duration of immobility was greater than on day 1 during the first and second minutes. The duration of immobility (seconds m ± S.D.) for the two first minutes was as follows: $(n-3)^-$ diet , first day 25.8 ± 25 (n=36); second day 42.3 ± 35. $(n-3)^+$ diet: first day 22.7 ± 23 (n=30); second day 57.9 ± 35. The duration of immobility increased between days 1 and 2 for the two groups; however, the effect was greater (255%) in the $(n-3)^+$ group than in the $(n-3)^-$ group (163%).

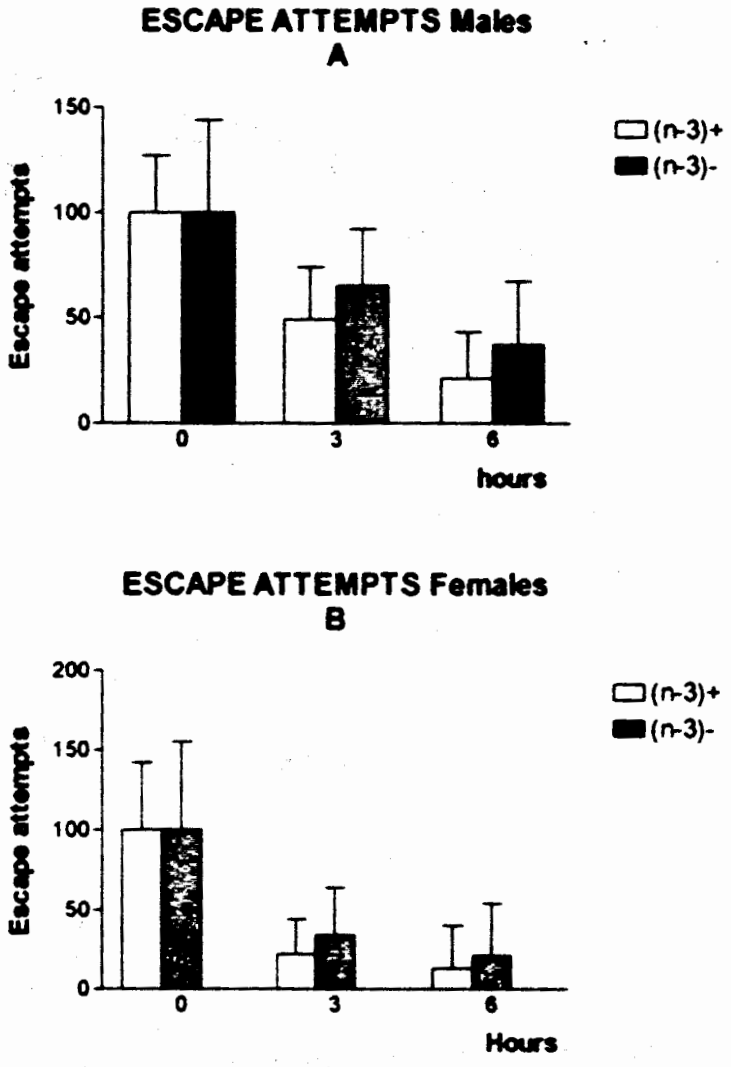

Fig. 3

A (males) and B (females). The mean number (± S.D.) of escape attempts recorded for 2 minutes was expressed as a percentage of the respective scores at time 0. 13 mice were used for each diet. The repeated measures analysis showed an effect of sex on the curve profiles (Huynh-Feldt $\varepsilon = 0.956$, $F_{2\varepsilon,\ 96\varepsilon} = 3.62$, $p=0.033$). The difference between the scores at 0 and 3 hours was significantly higher for females than for males ($t=2.27$, $df=48$, $p=0.028$ two-sided test). The effect of the diet on the curve profiles was borderline significant (Huynh-Feldt ε test $= 0.956$, $F_{2\varepsilon,\ 96\varepsilon} = 2.934$, $p=0.061$).

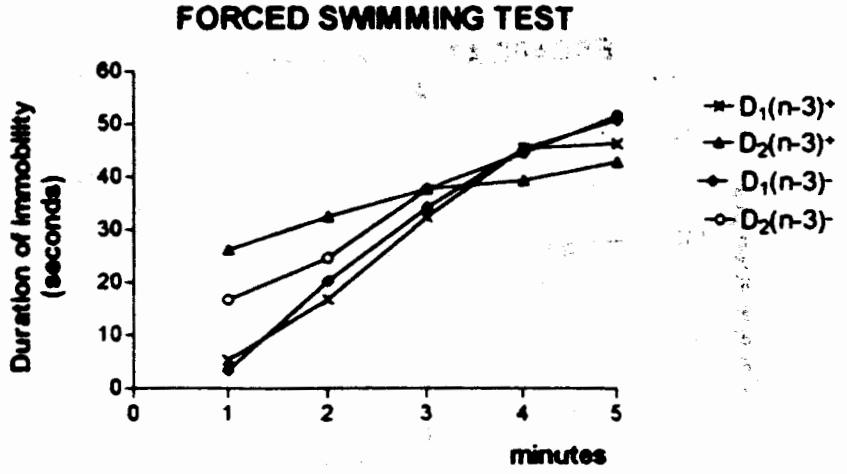

Fig. 4

The duration of immobility (seconds , means) is shown for 36 mice in the $(n-3)^-$ group and 30 mice in the $(n-3)^+$ group

A repeated measures analysis at Day 1 showed no influence of diets on either the profile of the curves or their levels during the 5 minute test

A repeated measures analysis with within-group factors day and time (limited to the first 2 minutes) showed a significant diet x day interaction ($F_{1,64}$= 5 22, p=0 026) The differences between the scores on day 2 and day 1 were significantly higher in the control group than in the deficient one for both the first minute (t=2 28, df=64, p=0 032, one-sided test).

Discussion

The results of the present study show that dietary restriction of n-3 fatty acids from three weeks before conception until sexual maturity markedly alters forebrain fatty acid composition, but does not significantly modify baseline activity of mice though it reduces their speed of habituation.

Results of the biochemical analysis show that in mice fed the $(n-3)^-$ diet, the major alteration in forebrain total fatty acid composition was a pronounced reduction in the content of 22 6(n-3) compensated for by an increase in the (n-6) fatty acids, 22 5 (n-6) and to a lesser extent 22 4 (n-6) and 20 4 (n-6) These results are in keeping with previous biochemical data obtained in rats fed a (n-3) deficient diet (1,3) , they suggest that the behavioral modifications may result from the modified levels of brain fatty acids

Several studies have investigated the effect of a reduced intake of n-3 fatty acids on the general activity of rodents. Yehuda (8) did not observe any change in the motor activity of rats placed for 15 min in a novel environment following 21 days of diet Bourre et al (1) did not find any change either in the motor activity recorded for one hour in a novel environment or in the openfield exploratory activity observed for 5 min in rats fed a deficient diet for three generations. For Enslen et al. (2), dietary restriction of n-3 fatty acids in rats, beginning in the dams 6 weeks before mating, decreased exploratory activity (at 60 min) in a novel environment without modifying spontaneous

locomotor activity (at 12 h after 17 h habituation). The present results fit well with those of Bourre et al. (1), Enslen et al. (2) and Yehuda (8) for spontaneous locomotor activity.

Regarding exploratory behavior, our results differed according to the model : if the level of exploration may be inferred from the score during the first 5 min in a novel environment, then our results indicate that neither the actimeter nor the water-filled beaker tests show any difference in activity in mice submitted to dietary restriction of n-3 fatty acids , in agreement with previous results from our laboratory obtained in rats (1). In the open-field test, the exploratory activity of (n-3) deficient mice, as measured by the number of squares crossed, was higher than that of (n-3) control mice ; this result disagrees with that of Enslen et al.(2) ; these different data may result from the duration of the test since the exploratory behavior recorded during 60 min may have different implications than that recorded during 5 min. Alternatively, some other parameters such as the species and the experimental conditions may be responsible.In addition, the rats of Enslen et al (2) were housed one per cage whereas our mice were housed 5-7 per cage and it is known that isolation can increase reactivity to novel situations (9,10). Therefore it is possible that the decreased openfield exploratory activity of rats fed a diet deficient in alpha-linolenic acid results from an interaction between the diet, the species and the isolated housing.

The number of rearings in the open-field did not differ between the two diets on either the first or second day.In the open-field test, the number of square crossings and of rearings are considered complex measures of both exploratory activity and emotional reactivity, whereas defecation appears more simply an index of emotionality. So, the lack of difference between the two diets for frequency of defecation on the first and second day seems to indicate that they do not induce different states of emotionality. A similar result had been previously obtained in rats (1). The lack of difference between the two diets for reactivity to noise may be due either to an absence of difference in reactivity or may result from a lack of significance of such a noise for the mice.

An influence of dietary α-linolenate deficiency on learning ability has been extensively studied. Using models of visual (4) and brightness (3,11) discrimination it has been shown that a diet enriched in α-linolenic acid increases the learning ability of mice and rats. Spatial learning in the Morris water maze (12) and active avoidance in a shuttle-box (1) give better results in rats fed a diet enriched in α-linolenic acid.

Although results regularly indicate a beneficial effect of the $(n-3)^+$ diet, interpretation remains difficult. Wainwright (5) reviewed all the difficulties including the effect of a deficiency on retinal function or pain threshold (8). Our results involve models in which the visual function and the pain threshold are not important. The simple form of learning that we have chosen (habituation) differs from more complex learning tasks such as instrumental and operant conditioning or spatial learning; in addition, the effect of dietary fat on habituation in rodents does not seem to have been studied.

In the actimeter, the exploratory activity measured for the first 5 min decreased significantly on the second test in the $(n-3)^+$ group only; this is an indication that these mice remember the situation better than the mice fed the $(n-3)^-$ diet. In the open-field, there was a decrease in the score of the mice fed the $(n-3)^+$ diet and not in the score of the mice fed the $(n-3)^-$ diet ; however, this trend of better recall in the $(n-3)^+$control group was not significant.

The forced swimming test is generally used as a screening test for antidepressant drugs (13) It is based on the idea that after exploration the animal understands that there is no escape, so the immobile posture reflects despair. Indeed, antidepressant drugs reduce the duration of immobility. However, a neurobehavioral study of forced swimming by West (14) indicated that immobility during forced swimming may not be a failure of coping but instead reflects a successful coping strategy that employs energy conserving behavior. The same author reports that the increased immobi-

lity on the second water exposure may be explained as learned habituation to a familiar environment so that this model may be used as a model of habituation. In our study, the duration of immobility for the two first min of forced swimming was greater on the second than on the first day in each diet, this means that the two groups had learned the situation, however, the effect was greater in the $(n\text{-}3)^+$ group indicating a better learning in this group.

The escape attempt model was first described by Frances (15). It was used to compare the reactivity of isolated and non-isolated mice. For naive mice, the beaker represents a new hostile place because there is neither food nor litter. It is thought that exploration represents an attempt to escape. Since escape is impossible, the best coping strategy for mice is (as in the forced swimming test) to reduce energy expenditure by reducing the number of escape attempts. So the reduction in the number of escape attempts with repetitive tests reflects learning. In our escape attempt tests, habituation led to a decrease in the score in both diet groups and for both sexes . However, the decrease was more rapid in the $(n\text{-}3)^+$ group for both males and females. Therefore it may be concluded that habituation is more rapid with the $(n\text{-}3)^+$ diet than with the $(n\text{-}3)^-$ diet. In addition, this model showed a more rapid habituation in females than in males.

The reasons for the difference in habituation between (n-3) deficient and non-deficient mice are unknown. Several possibilities may be proposed: an effect on adult CNS function - an effect on maternal health which could affect gestation or maternal behavior nonspecifically before weaning - or an effect on fetal or neonatal development. Some differences in maternal behavior or neonatal development cannot be excluded since these were not extensively studied as they were not the aim of the present experiments. However, the lack of marked differences in the fecundity, fertility, and weight of the pups at 22, 47 and 61 days of age is in accordance with the findings of Guesnet, Pascal and Durand (16) and Lamptey & Walker (17) in rats. Further, no perinatal mortality was observed in our mice ; this is in contrast with the results of Guesnet et al. (16) in rats but may be explained because the rats used by these authors were fed a diet deficient in (n-3) PUFA for 3 generations.

Consequently, there are no obvious reasons to ascribe the slower habituation of (n-3) deficient mice to a problem of maternal health or care or to a defect in neonatal development. More likely, changes in CNS function related to modifications in PUFA are involved. Are alterations of particular brain structures linked to modification of particular forms of behavior ? A decrease in the density of dopamine D2 receptors and an increase in the density of serotonin 5-HT2 receptors have been measured in the frontal cortex of rats fed a diet chronically deficient in alpha-linolenic acid (18) These local modifications may alter the behavior and are not exclusive with more general changes such as, for example, an alteration in the speed of neurotransmission which could take place everywhere in the brain and be responsible for multiple forms of behavioral (or pharmacological) changes.

In conclusion, these experiments using models that do not involve pain or restriction of food or water to motivate performance demonstrate that a diet deficient in α-linolenic acid reduces the learning ability of mice in various models of a simple form of learning: habituation. These results add fresh data to those previously obtained in different laboratories with other behavioral models.

Acknowledgements

This work was supported by the Institut National de la Santé et de la Recherche Médicale

References

1 J M. BOURRE, M. FRANCOIS, A. YOUYOU, O DUMONT, M. PICIOTTI, G. PASCAL and G. DURAND, J. Nutr. 119 1880-1892 (1989).
2 M. ENSLEN, H. MILON and A. MALNOE, Lipids 26 203-208 (1991).
3 N. YAMAMOTO, M. SAITOH, A. MORIUCHI, M. NOMURA and H. OKUYAMA, J. of Lipid Res. 28 144-151 (1987).
4 H. FRANCES, C. MONIER and J.M. BOURRE, Life Sci. *in press*
5 P. WAINWRIGHT, Neurosci. and Biobehav. Reviews 16 193-205 (1992).
6 G. LEPAGE and C. ROY, J.Lipid Res. 27 114-120 (1986).
7 D.F. MORRISSON Multivariate statistical methods, Chap. 5, McGraw-Hill (ed.) 236-247, New-York (1976)
8 S. YEHUDA, Intern. J. Neurosci. 32 919-925 (1987)
9 H. FRANCES, C. LIENARD, J. FERMANIAN and Y. LECRUBIER, Pharmacol. Biochem. Behav. 32 637-642.
10 L. VALZELLI Handbook of Psychopharmacology vol 7 L.L. Iversen, S.D. Iversen and S.H. Snyder (Eds), 369-392 New-York (1977)
11 S. YOSHIDA, A. YASUDA, H. KAWASATO, K. SAKAI, T. SHIMADA, M. TAKESHITA, S. YUASA, Y. FUKAMIZU and H. OKUYAMA, Advances in polyunsaturated fatty acid research T. Yasugi, H. Nakamura and M. Soma (Eds). 265-268, Elsevier Science Publishers B.V., (1993).
12 Y. NAKASHIMA, S. YUASA, Y. HUKAMIZU, H. OKUYAMA, T. OHHARA, T. KAMEYAMA and T. NABESHIMA, J. Lipid Res. 34 239-247 (1993)
13 R. PORSOLT, G. ANTON, N. BLAVET and M. JALFRE, Eur. J. Pharmacol. 47 379-391 (1978)
14 A.P. WEST, Prog. Neuro-Psychopharmacol. and Biol. Psychiat. 14 863-877 (1990).
15 H. FRANCES, Pharmacol. Biochem. Behav. 29 467-470 (1988)
16 P. GUESNET, G. PASCAL and G. DURAND Reproduction Nutrition Development 26 969-985 (1986).
17 M.S. LAMPTEY and B.L. WALKER, J. of Nutrition 108 358-367 (1978)
18 S. DELION, S. CHALON, J. HERAULT, D. GUILLOTEAU, J.-C. BESNARD and G. DURAND, J. Nutr. 124 2466-2476 (1994)