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Effect of Threonine on the Behavioural Development of the Rat

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CASTAGNE, V., J.-C. MAIRE, D. MOENNOZ AND M. GYGER. *Effect of threonine on the behavioural development of the rat.* PHARMACOL BIOCHEM BEHAV **52**(2) 281–289, 1995.—Rats received different levels of threonine (Thr), one, 1.7, and four times the normal dietary intake, from conception to adulthood. The mothers were fed the experimental diets before and during pregnancy. Their offspring received a daily oral load of Thr or placebo until weaning. Thereafter, the juveniles were fed the same diet as their mothers. Morphologic development, ingestive behaviour, homing, and locomotion were observed before weaning. Exploration and spontaneous alternation were studied thereafter. Animals exposed during gestation to 1.7 times the normal Thr intake consumed more food during the test of independent ingestion. Grooming showed inconsistent variations between days 12 and 29 in pups fed 1.7 times the normal Thr intake. Rats performed equally well on the other behavioural tasks independently of the dietary treatment. We conclude that Thr intake as much as four times higher than the levels found in normal diets does not impair the behavioural ontogenesis of the rat.

Rat Behaviour Ontogenesis Threonine Amino acid Brain Locomotion Homing Feeding

AMINO acid imbalances may have dramatic consequences on the CNS. Inadequacies in certain types of amino acid intake (e.g., phenylalanine or tryptophan) have irreversible effects on neurologic development (20). In adult humans, large dietary threonine (Thr) supplementation has been used as a tentative treatment of spasticity (3,15) and has been used unsuccessfully for amyotrophic lateral sclerosis (4). Threoninemia is a metabolic disease that has been described in an 8-mo-old boy (30). Serum Thr levels were seven times higher than the normal range, and urinary Thr output was also increased. This defect in Thr metabolism was associated with convulsions and growth perturbation. The Thr dietary intake of infants has been questioned by pediatricians (31). It is generally assumed that cow milk protein-based infant formulae contain a moderate excess of Thr as compared to human milk and induce an elevation of plasma Thr levels whose developmental consequences are difficult to evaluate (32). A large excess of dietary Thr has been shown to be only weakly toxic for the rat in comparison with other amino acids (34). Nevertheless, the chronic effects of high Thr intake on the rat's development have not been extensively studied, and data are still lacking for the preweaning period, which is very important for brain maturation (12).

Excess Thr may impair brain functions through several mechanisms. In the liver, Thr is partly converted into glycine (Gly) (7), and from Gly into serine (Ser). After release into the blood circulation, Gly and Ser may reach the brain. Gly is a neurotransmitter (2) whose excess has dramatic effects on the neonatal brain (11). Nonketotic hyperglycinemia (NKH) is a metabolic disease characterized by large elevations of tissue Gly levels and dramatic neurologic disorders induced by the rise of brain Gly concentrations (10,36). In the rat, it is possible to reproduce neurologic abnormalities characteristic of NKH by elevating brain Gly levels (11). Ser modulates glutamate (Glu) receptors (33), which have important neurologic functions (28). In particular, Glu neurotransmission is critically involved in brain development (26), because Glu influences neuronal survival (14) and migration (23), dendritic arborization (6), and synaptogenesis (29), as well as plasticity in large brain regions such as the visual system (22). Thr competes with other amino acids for transport through the blood-brain barrier (37). Consequently, a rise in blood Thr levels may lower the uptake of other amino acids into the brain, leading to a drop in the synthesis of some neurotransmitters such as serotonin and catecholamines (19), and histamine (27). Moreover, a decrease in amino

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acid availability may lead to a decrease in brain protein synthesis (25).

We demonstrated that diet-induced hyperthreoninemia barely elevates brain levels of free Gly and Ser (7) and does not modify the extracellular levels of Gly, Ser, aspartate, and Glu (8), suggesting that brain neurochemistry is unaltered in Thr-loaded rats. Nevertheless, these studies were done on rats with already matured brains. The aim of the present study was to describe the neurobiologic consequences of diet-induced hyperthreoninemia during the entire period of brain maturation. To assess this, behavioural and morphologic analyses were done from birth to 3 mo of age.

METHOD

Animals and Diets

Twelve female Sprague-Dawley rats (Iffa Credo, Lyon, France) intended for breeding were housed individually under environmentally controlled conditions (12 L : 12 D cycle with lights on from 0630 to 1830 h, with a temperature of 23 ± 2°C and relative humidity 55 ± 10%). Rats were fed a standard diet (NAFAG 890, Gossau, Switzerland) until 1 week before mating. Thereafter, mothers were randomly assigned to one of four groups until the end of lactation: group 1, fed diet A (NAFAG 850), containing 0.96 g of Thr/100 g dry matter; groups 2 and 3, fed diet B (99.33% NAFAG 850 + 0.67% L-Thr), 1.63 g of Thr/100 g; and group 4, fed diet C (97.12% NAFAG 850 + 2.88% L-Thr), 3.84 g of Thr/100 g.

Each mother was mated with one male during the course of 8 days. The day of birth of the offspring was defined as day 1. Three litters, culled to eight pups, were assigned to each experimental group. In each litter, four pups (two males and two females) were selected for the study. Therefore, each group contained 12 pups (six males and six females). Beginning on day 2 and until weaning, pups in groups 1 and 2 received an oral infusion of water, whereas pups in group 3 received a moderate supplement of L-Thr [calculated to be around 1.7 times the normal daily Thr intake through mother's milk (9)]. Pups in group 4 received a high supplement (four times the normal intake). On day 21, pups were weaned and housed in cages of two males or two females. At weaning, oral infusions were replaced by a solid diet similar to the one given to the mothers. On day 50, NAFAG 850 (24% protein) was replaced by NAFAG 890 (18% protein), because of the lesser protein requirements of the rat after the rapid growth period. Diet composition was modified as follows: group 1, fed diet A (NAFAG 890), containing 0.68 g of Thr/100 g dry matter; groups 2 and 3, fed diet B (99.52% NAFAG 890 + 0.48% L-Thr), 1.16 g of Thr/100 g; and group 4, fed diet C (97.96% NAFAG 890 + 2.04% L-Thr), 2.72 g of Thr/100 g.

Group 1 was the control group. Pups of group 2 were exposed to a moderate supplement of Thr (1.7 times that of group 1) during gestation and after weaning only. Pups of groups 3 and 4 received Thr supplements from conception to adulthood, at moderate (1.7 times those of group 1 for group 3) and high levels of Thr (four times those of group 1 for group 4). These supplements are comparable to the supplements used previously for neurochemical studies (7,8).

Pups were weighed every 4 days between days 2 and 22 and weekly thereafter. Food intake during 24 h was assessed weekly for each cage of two animals from day 30 until sacrifice.

Rats were decapitated on day 90 after a normal nighttime feeding period. Brain, liver, kidneys, and testes were weighed. Trunk blood was sampled according to (7). Plasma amino acid levels were measured by HPLC (PICO-TAG method; Waters).

Behaviour

To evaluate the behavioural ontogenesis at different ages, five tests were selected. At an early age, ingestive behaviour, homing, and locomotion were measured. Independent ingestive behaviour was studied at day 6 and homing at days 9 and 10. Locomotion was monitored every 2 days between days 12 and 20. To observe eventual later consequences of early perturbations, two classical behavioural tests were used: Open-field activity was measured on days 29 and 70, and spontaneous alternation during four age periods (between days 21 and 23, days 42 and 44, days 63 and 65, and days 84 and 86). All observations were made during the light period of the daily cycle.

Independent ingestive behaviour. Pups were deprived of their mother for 3 h by placing them with their littermates in a temperature-controlled environment (33 ± 1°C). The test chamber had its floor covered with a 10% saccharose solution (16) and the chamber temperature was adjusted to 36 ± 1°C. Pups could ingest saccharose during 30 min, with intake being defined as the pup's weight difference before and after testing. Care was taken to stimulate the anogenital region before testing, allowing the pup to urinate and defecate. Relative food intake was calculated as the percentage change of the initial body weight.

Homing. The four littermates were simultaneously tested in the multipup homing apparatus (38). For testing, the mother was placed in the goalbox and pup's behaviour was observed over 180 s. The latency to leave the start area, the latency to enter the goal area, and the duration of homing (defined as the presence of the pup in the goal area) were recorded. To distinguish homing from other behaviours, pups were tested twice a day, once with the mother and once without.

Locomotion. Pups were individually tested in an elongated runway (100 × 15× 20 cm) (39). Black lines allowed the measure of locomotor activity. For 120 s, the number of lines crossed, number of rearings, and duration of adultlike locomotion and grooming were measured. Adultlike locomotion was defined as walking with the body's ventral face held above the floor.

Open-field activity. Rats were tested in a well-illuminated open field (112 × 112 cm) divided into 4 × 4 squares (28 × 28 cm). Rats were placed in the centre of the open field and observed for 300 s. The number of lines crossed in periphery and in the centre of the area, and the number of rearings were scored. The duration of grooming and the number of droppings were also measured.

Spontaneous alternation. The subjects were observed during four periods in a Y-maze with cross arms making a 60° angle with the main alley. Arms and alley were 40 cm long, 13 cm wide, and 20 cm high. The first 10 cm of the main alley constituted the start area, separated from the rest of the maze by a sliding door. After 10 s in the start area, the door to the alley was raised. A right or left score was recorded when all four of the rat's feet were completely in one cross arm. The pup was then restricted to the arm for another 10 s. The next trial started after the rat was placed in the start box again for 10 s. Each period of testing included 3 days of observation, each day included two trials allowing the rat to alternate once

TABLE 1

MEAN BODY (g) AND ORGANS (% OF BODY) WEIGHTS ± SEM ON DAY 90

		Group 1 (n = 6 M, 6 F)	Group 2 (n = 6 M, 6 F)	Group 3 (n = 6 M, 6 F)	Group 4 (n = 7 M, 5 F)
Body 2	M	7.73 ± 0.18	7.65 ± 0.21	6.77 ± 0.17*†	6.39 ± 0.21*†
	F	7.57 ± 0.16	7.23 ± 0.32	6.43 ± 0.16*†	5.92 ± 0.14*†‡
Body 90	M	522 ± 16	553 ± 22	481 ± 13†	461 ± 9*†
	F	326 ± 9	304 ± 9	294 ± 6*	279 ± 4*†
Brain	M	0.39 ± 0.01	0.36 ± 0.01	0.41 ± 0.02	0.42 ± 0.01*
	F	0.58 ± 0.01	0.60 ± 0.02	0.64 ± 0.02*	0.62 ± 0.01*
Kidney	M	0.73 ± 0.03	0.67 ± 0.02	0.66 ± 0.03	0.69 ± 0.03
	F	0.72 ± 0.03	0.67 ± 0.02	0.63 ± 0.02*†	0.70 ± 0.02‡
Liver	M	4.20 ± 0.08	4.12 ± 0.07	4.24 ± 0.19	3.87 ± 0.10
	F	3.73 ± 0.17	3.60 ± 0.07	3.57 ± 0.13	3.54 ± 0.12
Testes	M	0.80 ± 0.03	0.74 ± 0.03	0.83 ± 0.06	0.83 ± 0.04

Body 2: body weight at day 2; Body 90: body weight at day 90. M: Males; F: females.
*Different from group 1, $p < 0.05$, MW tests.
†Different from group 2, $p < 0.05$.
‡Different from group 3, $p < 0.05$.

a day. For each period, the total number of alternation could range from 0–3 (21).

Statistics

Because four rats were chosen in each litter, the homogeneity of the population within each experimental group (three litters, 12 pups) was tested (1). A Kruskal–Wallis one-way analysis of variance (KW ANOVA) was performed for each parameter measured and for each experimental group (35). The membership to a litter had no effect on the performances of pups. As a consequence, the factor Litter was omitted in later analysis.

Data were analysed by two-factor (Treatment × Sex) or three-factor ANOVA with repeated values for day (Treatment × Sex × Day). If an effect of sex was found, data of males and females were analysed separately. Thereafter, the effect of treatment was analysed by KW ANOVA followed by Mann–Whitney tests (MW tests). A difference was considered significant at $p < 0.05$. For homing, data were subjected to four-factor ANOVA with repeated values for day (Treatment × Sex × Mother × Day) to analyse the effect of the mother. Data with and without the mother were then analysed separately by three-factor ANOVA (Treatment × Sex × Day). Treatment effect was studied by KW and MW tests. For spontaneous alternation, the probability of right and left turns on initial trials was calculated. Alternation number was tested against chance by χ^2 test.

RESULTS

Morphologic Parameters

Diets did not influence the time of apparition of fur and incisors, or the time of ears and eyes opening. Body weight

TABLE 2

PLASMA AMINO ACID LEVELS (μM) ± SEM

	Group 1 (n = 6 M, 6 F)	Group 2 (n = 6 M, 6 F)	Group 3 (n = 6 M, 6 F)	Group 4 (n = 7 M, 5 F)
Thr				
M	294.2 ± 11.5	497.4 ± 62.0*	420.6 ± 43.4*	1121.1 ± 111.8†‡§
F	423.4 ± 42.1	617.0 ± 73.9*	600.8 ± 36.6*	1224.9 ± 157.6†‡§
Gly				
M	331.8 ± 38.5	347.3 ± 34.2	357.5 ± 33.5	394.4 ± 25.8*
F	300.6 ± 15.1	371.7 ± 36.5	342.3 ± 28.9	408.2 ± 35.9*
Ser				
M	258.5 ± 7.7	254.6 ± 10.3	246.8 ± 4.9	242.0 ± 10.2
F	284.3 ± 21.2	267.8 ± 9.2	268.8 ± 17.5	291.0 ± 18.4

M: Males; F: females.
*Different from group 1, $p < 0.05$, MW tests.
†Different from group 1, $p < 0.01$.
‡Different from group 2, $p < 0.01$.
§Different from group 3, $p < 0.01$.

(Table 1) was modified by diets ($F(3, 47) = 17.131$, $p < 0.001$), sex ($F(1, 47) = 392.67$, $p < 0.001$), and age ($F(18, 864) = 8372.9$, $p < 0.001$). Mother's body weight was not modified by diets. In males, diets modified body weight ($F(3, 24) = 11.888$, $p < 0.001$); this effect was observed every day [KW ANOVA, H(3) ranged between 11.349 and 18.953, $p < 0.01$]. Diets influenced females' body weight ($F(3, 22) = 14.384$, $p < 0.001$) at all times of measurement [KW ANOVA, H(3) was between 11.028 and 18.377, $p < 0.05$]. Food intake, measured weekly for a period of 24 h between days 30 and 79, did not vary significantly between treatments.

Brain and liver weights, expressed as a percent of body weight (relative weight), were different in males and females [$F(1, 47)$ was between 40.186 and 518.081, $p < 0.001$; Table 1]. Other organ-relative weights were not different between sexes. In males, diet modified the relative brain weight [KW ANOVA, H(3) = 10.159, $p < 0.05$]. Other organ weights, including testes, were not modified by diet. In females, dietary treatment modified relative brain [KW ANOVA, H(3) = 8.115, $p < 0.05$] and kidney [KW ANOVA, H(3) = 11.223, $p < 0.05$) weights.

Plasma Amino Acid Levels

Thr levels were modified by sex ($F(1, 47) = 6.889$, $p < 0.05$; Table 2). Diet effect was important in males [KW ANOVA, H(3) = 20.120, $p < 0.001$) and females [KW ANOVA, H(3) = 15.576, $p < 0.01$). Diet B moderately elevated Thr levels in both groups 2 and 3, and diet C strongly raised the threoninemia in group 4. Gly levels were modified by dietary treatment ($F(3, 47) = 2.868$, $p < 0.05$). Rats in group 4 had higher Gly levels than rats in group 1.

Behaviour

Independent ingestive behaviour. Food intake was modified by treatment ($F(3, 47) = 5.878$, $p < 0.01$; KW ANOVA, H(3) = 11.156, $p < 0.01$; Fig. 1] but not by sex. Rats of group 2 (mother fed 1.7 times the Thr content of diet A and pups receiving no subsequent Thr supplement) consumed more saccharose than the other groups. Relative food intake was modified by dietary treatment ($F(3, 47) = 4.572$, $p < 0.01$; KW ANOVA, H(3) = 11.074, $p < 0.05$). Rats from group 2 had a higher relative food intake than rats of groups 1 and 3.

Homing. The mother diminished the latency to start on day 10 ($F(1, 48) = 13.531$, $p < 0.001$) but not on day 9. The latency to goal was diminished by the presence of the mother ($F(1, 48) = 6.336$, $p < 0.05$ and $F(1, 48) = 14.800$, $p < 0.001$, respectively, on days 9 and 10]. Homing duration was raised by the mother ($F(1, 48) = 17.950$, $p < 0.001$ and $F(1, 48) = 49.444$, $p < 0.001$ on days 9 and 10, respectively], illustrating the specificity of the test (38).

Diet modified the latency to start in the absence of the mother [H(3) = 20.328, $p < 0.001$ and H(3) = 8.010, $p < 0.05$ for days 9 and 10, respectively; not shown]. On day 9 but not on day 10, rats in group 4 had a shorter start latency than the other groups. The latency to goal and homing duration were not modified. In the presence of the mother, diet had no effect (Fig. 2).

Locomotion. The number of lines crossed rose from days 12-20 ($F(4, 192) = 91.473$, $p < 0.001$); sex and treatment had no effect (Fig. 3). Adultlike walking appeared as pups matured ($F(4, 192) = 110.828$, $p < 0.001$). The number of rearings was very sensitive to age ($F(4, 192) = 136.754$, $p < 0.001$) but not to sex or dietary treatment. Grooming increased from days 12-20 ($F(4, 192) = 37.657$, $p < 0.001$). Diet influenced grooming ($F(3, 47) = 2.896$, $p < 0.05$) on days 12 and 18 [KW ANOVA, H(3) = 7.973, $p < 0.05$ and H(3) = 12.328, $p < 0.01$, respectively]. On day 12, rats in group 2 groomed less than rats in group 1. On day 18, rats of group 3 and 4 groomed more than rats in groups 1 and 2.

Open-field activity. On day 29, sex had no effect and dietary treatment did not modify the parameters studied, except for grooming [KW ANOVA, H(3) = 8.919, $p < 0.05$]. Rats of group 3 groomed more than rats in groups 1 and 2 (Fig. 4). On day 70 (results not shown), the defecation score was zero for all animals, except for one male in group 2. Sex influenced

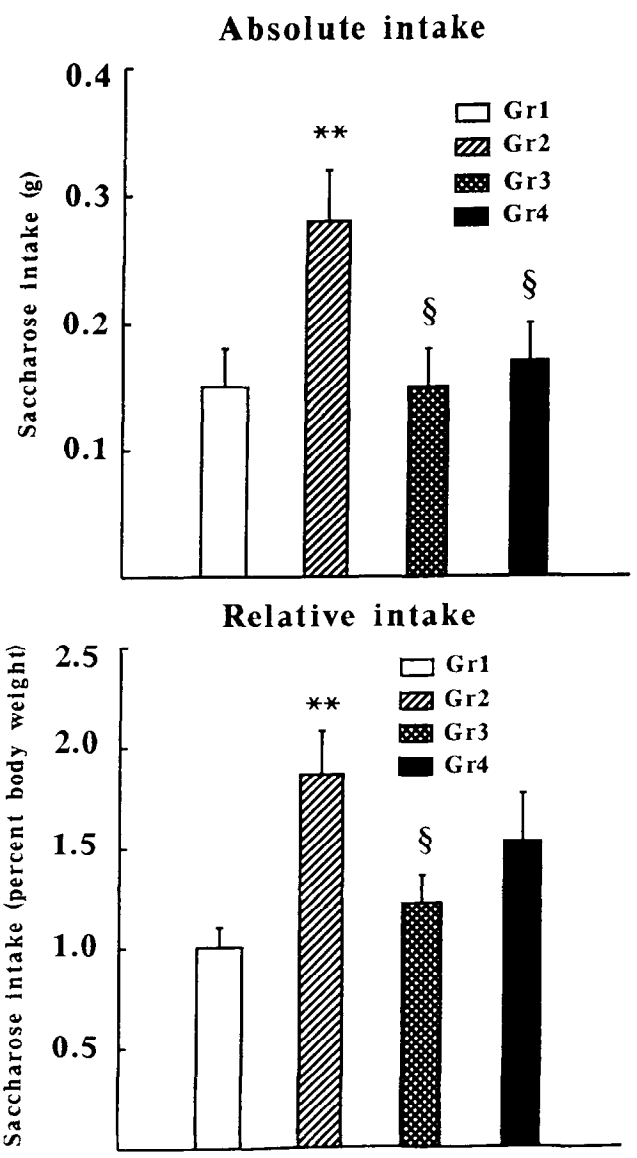

FIG. 1. Independent ingestive behaviour on day 6. Absolute (g) and relative (percent of body weight) intake of saccharose ± SEM during 30 min. **Different from group 1, $p < 0.01$; §different from group 2, $p < 0.05$, MW tests.

the number of lines crossed in the periphery and in the centre of the field, and the number of rearings [KW ANOVA, H(1) = 18.181, $p < 0.001$, H(1) = 9.717, $p < 0.01$, and H(1) = 13.925, $p < 0.001$, respectively]. For these three parameters, females had higher scores than males. Rearings were modified by diet [KW ANOVA, H(3) = 11.351, $p < 0.01$], with rats in group 4 rearing more than groups 1 and 2.

Spontaneous alternation. Rats did not display any significant bias on initial arm choice. The number of alternation developed gradually with time [$F(3, 144) = 24.122$, $p < 0.001$; Fig. 5). On the first session (rats 21–23 days old), the number of alternations during the 3 consecutive days was 1.06 ± 0.13 (35% alternation rate) and was below chance level [$\chi(3) = 8.43$, $p < 0.05$]. On days 42–44, alternation rose to 1.33 ± 0.15 (44%, not different from chance). Significant alternation appeared on days 63–65 with 2.15 ± 0.12 alternations, which is above the chance level (72%, $\chi(3) = 12.64$, $p < 0.01$). On days 84–86, alternation rose further to 2.25 ± 0.12 [75%, $\chi(3) = 17.55$, $p < 0.001$). Sex and diet did not modify alternation.

DISCUSSION

The diets clearly increased plasma Thr levels. As compared to group 1, rats in group 2 were exposed to high Thr intake (1.4–1.7 times basal levels in group 1) during gestation and after weaning. Rats in group 3 received the same Thr intake as in group 2 during the whole 90-day study and gestation. Rats in group 4 were exposed to a very high Thr intake (2.9–3.8 times basal levels) during the whole study. In agreement with previous studies (7), the glycinemia was elevated by very high Thr intake (group 4) but not by a moderate intake (groups 2 and 3).

Morphologic Development.

Body weight on day 90 was reduced by large excess of Thr (group 4), in accordance with studies done in older rats (7,34). Rats in group 3, especially females, which received a moderate Thr supplement, also had a lower body weight than control rats. Nevertheless, this effect was also present on day 2, although groups 2 and 3 received the same Thr treatment at this time. The lower body weight of rats of group 3 on day 90 was probably a consequence of their paradoxically low birth weight. Group 2, which was not exposed to high Thr levels during suckling, did not differ from group 1. A moderate elevation of dietary Thr before and after the suckling period had no significant effect on the development of organs (comparison between groups 1 and 2). When treatment was extended to suckling (group 3), the relative kidney weight was diminished and brain relative weight increased in females. Relative brain weight was raised in group 4 and in the two sexes.

On the whole, our results show that a high Thr intake affects mainly body weight. Moreover, the fastest period of growth corresponding to suckling is more sensitive to Thr intake. Specific organ weight does not seem to be affected by Thr intake, with the exception of kidneys in females. The elevation of relative brain weights in groups 3 and 4 reflects the lower body weight of these animals at sacrifice. Because absolute brain weights were not elevated in these rats as com-

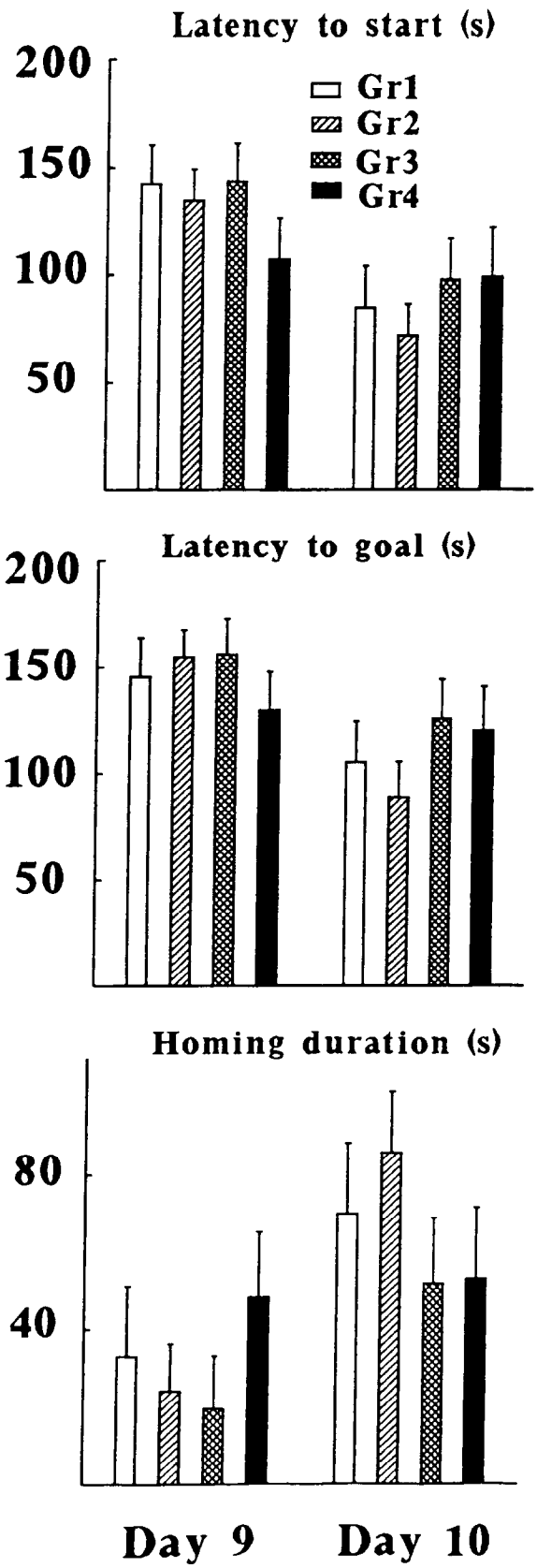

FIG. 2. Homing in the presence of the mother on days 9 and 10. Latencies to leave the start and to reach the goal and homing duration (s) ± SEM during 3 min.

pared to groups 1 and 2, our results suggest the absence of a large effect of Thr on cerebral development.

Behavioural development. Independent ingestive behaviour is the main precursor form of adult feeding patterns (17). Pups in group 2 (exposed to moderate Thr levels during the gestation but not after birth) consumed more saccharose than pups in group 1. The difference disappeared when Thr intake was elevated from day 2 to the day of testing (groups 3 and 4). A change in motivation to consume saccharose could arise from a lower quantity or quality of mothers' milk. Nevertheless, this seems unlikely, as rats in groups 3 and 4 consumed a similar quantity of saccharose as in group 1. There are presently no hypotheses involving Thr as the causative agent in this effect. However, all pups consumed a significant amount of saccharose whether they were exposed to Thr, suggesting that sensory, motivational, and motor processes involved in the regulation of independent ingestion (18) are not impaired by Thr supplementation.

Homing was not modified by dietary treatment when the mother was present. In her absence, pups in group 4 had a shorter latency to start than the other groups. This effect was not confirmed on day 10 and was not observed for the latency to goal or the duration of homing. Again, results showed no specific and systematic effect of Thr on a complex behaviour such as homing. The sensory processes involved in the recognition of the mother (olfactory and perhaps auditory) were

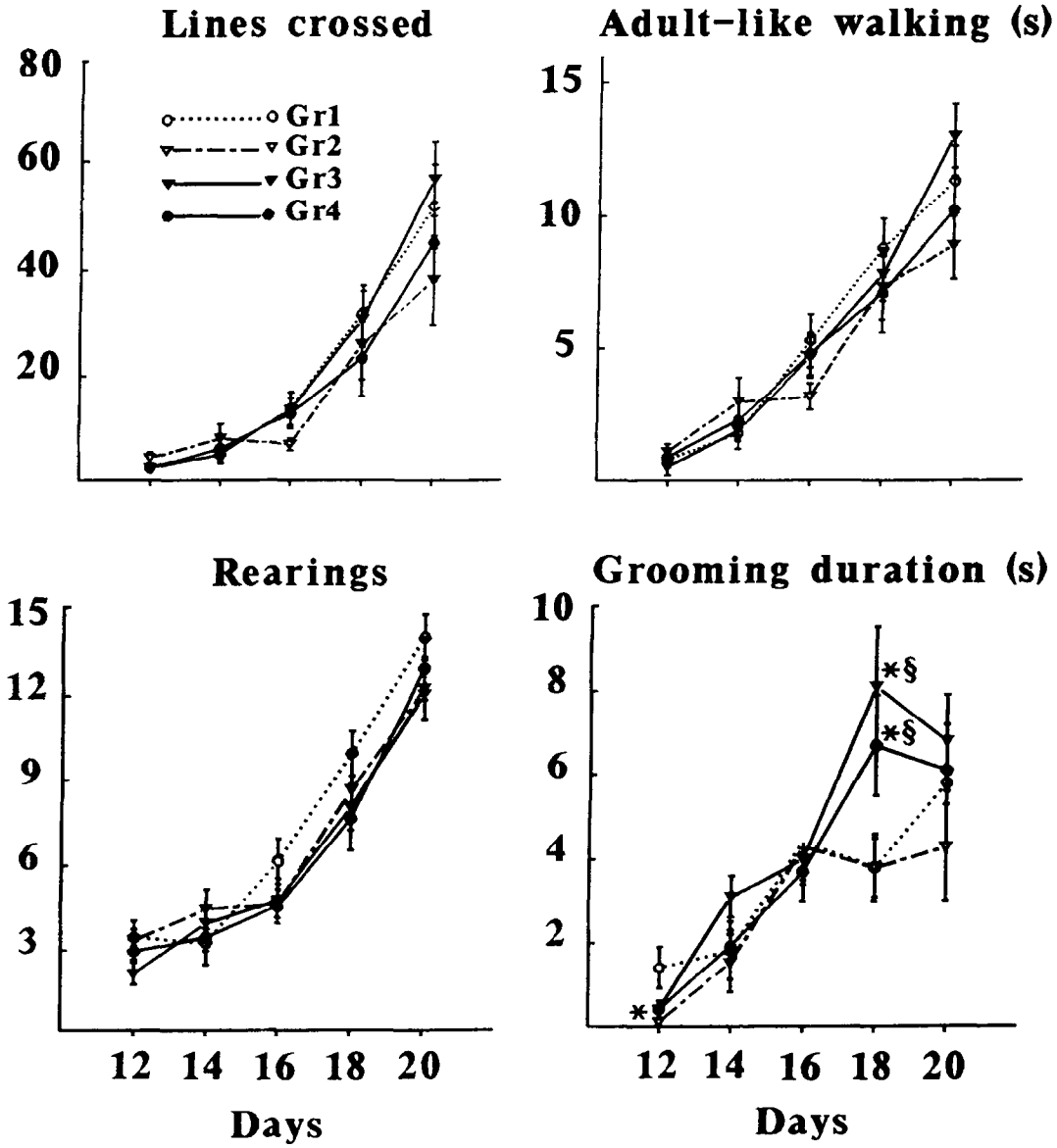

FIG. 3. Development of motor patterns from days 12–20. Number of lines crossed and of rearings ± SEM, and duration of adultlike walking and grooming (s) ± SEM during 2 min. *Different from group 1, $p < 0.05$; §different from group 2, $p < 0.05$, MW tests.

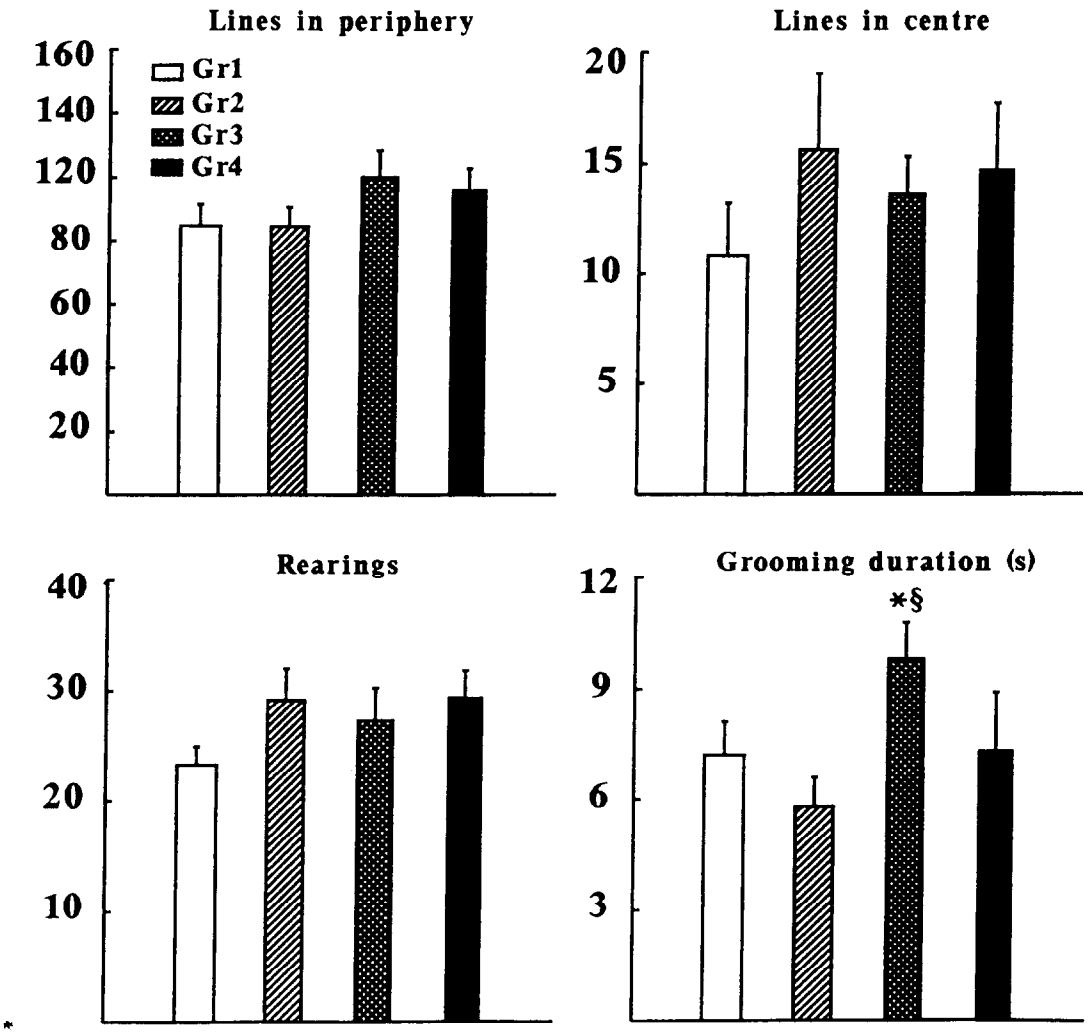

FIG. 4. Open-field activity on day 29. Number of lines crossed in the centre and in the periphery of the open field, and number of rearings ± SEM and grooming duration (s) ± SEM during 3 min. *Different from group 1, $p < 0.05$; §different from group 2, $p < 0.05$, MW tests.

equally developed in Thr supplemented pups (groups 2–4) as in group 1.

In accordance with published data (39), the duration of adultlike walking and the intensity of locomotor activity rose with age, whatever the Thr treatment. This is in line with the results of the other tests, showing that Thr does not impair motor skills in the developing rat. On day 12, rats in group 2 groomed less than group 1, whereas on day 18 rats in groups 3 and 4 groomed more than groups 1 and 2. The fact that there was not a clear pattern of differences in grooming levels suggests that Thr may have affected this behaviour only in a transitory manner. Moreover, it appears that the difference observed on day 18 could be explained by the low incidence of grooming behavior of rats in groups 1 and 2 rather than by an elevation of grooming activity in groups 3 and 4. This transitory diminution of grooming is in accordance with the irregular apparition of grooming behavior, which has already been described in extensive studies of rats' behavioral development (5).

With the exception of elevated grooming in group 3 on day 29 and of increased rearing in group 4 on day 70, all other parameters measured in the open field were unaffected by diets. The increase in grooming in group 3 does not seem to reflect a specific effect of Thr, because it was not found in groups 2 and 4, which also received Thr supplements, and was not apparent on day 70 in group 3. No systematic effect associated with Thr intake emerged from our results, indicating that diet did not modify emotionality as measured by the open-field test (40). This suggests a normal development of the limbic system known to be involved in the control of emotions (24).

Spontaneous alternation developed gradually with age, in agreement with other studies (13,21). Dietary treatment did not modify alternation, suggesting a normal ontogenesis of

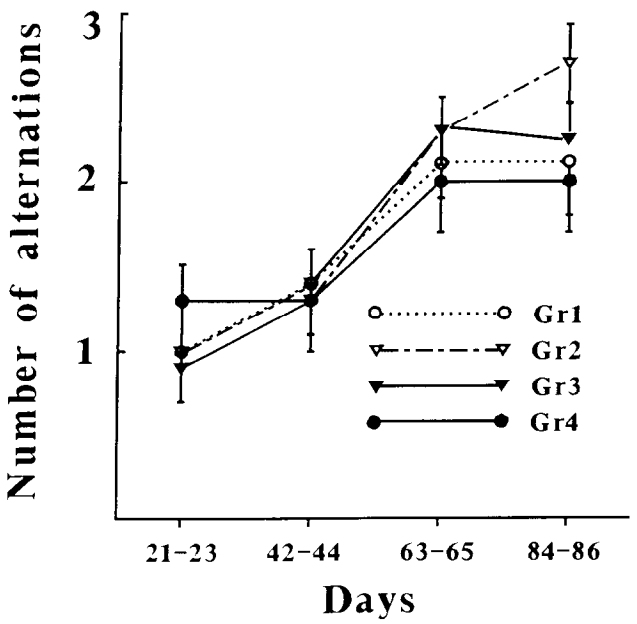

FIG. 5. Development of spontaneous alternation in a Y-maze from weaning to adulthood. Mean total number of alternations ± SEM during four 3-day sessions.

the structures involved in the processing of spatial cues, mainly the hippocampus, one of the latest structures of the brain to mature (13,21).

Conclusions

Supplementation with Thr in moderate (1.7 times the normal Thr intake) or high levels (four times normal) does not modify the ontogenesis of behaviour in the rat. Sensory processes are preserved; the motivational state appears normal with respect to ingestive and homing behaviours, as well as the motor skills involved in ingestion, homing, and locomotion. No effect of increased levels of Thr could be observed in an open field and Y-maze. In addition, there was no obvious alteration of the endocrine system as judged by changes in organ weights. These results, combined with previous neurochemical data (7,8), suggest that consumption of high levels of Thr do not impair the neurologic development of the rat.

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