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Effect of Chemically Induced Propionic Acidemia on Neurobehavioral Development of Rats

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BRUSQUE, A. M., C. F. MELLO, D. N. BUCHANAN, S. T. TERRACCIANO, M. P. ROCHA, C. R. VARGAS, C. M. D. WANNMACHER AND M. WAJNER. *Effect of chemically induced propionic acidemia on neurobehavioral development of rats.* PHARMACOL BIOCHEM BEHAV **64**(3) 529–534, 1999.—High levels of propionic acid (PPA) comparable to those of human propionic acidemia were achieved in blood (1–5 mmol/l) and brain (1 μmol/g) of rats by administering saline-buffered propionate (pH 7.4) subcutaneously twice a day from the 6th to the 28th day of life. PPA doses ranged from 1.44 to 1.92 μmol/g body weight as a function of animal age. Control rats were treated with saline in the same volumes. Growth and development of physical landmarks were assessed by monitoring the following parameters daily: body weight, upper incisor eruption, eye opening, and hair coat. Development of some reflexes was also monitored, and a specific subset of motor skills was evaluated at days 14 and 21 of life by the free-fall righting test and the spontaneous alternation test. Chronic PPA administration had no effect on body weight, cerebral cortex weight, or cerebellum weight, but caused slight but significant delays in the day of appearance of hair coat and eye opening, indicating an effect of PPA on the development of physical parameters. Free-fall righting was impaired in PPA-treated animals. On the other hand, PPA administration had no effect on the performance of the animals in the spontaneous alternation tests. Long-term effects of early PPA administration were investigated by assessing animal performance in an aversive task (two-way shuttle avoidance task) and in a nonaversive (open-field task) behavioral task at 60 days of age. PPA-treated rats did not habituate to the open field, and presented a lack of retention of the shuttle-avoidance task. Our results suggest that early postnatal PPA administration to rats alters normal development and induces long-term behavioral deficits in aversive and nonaversive tasks. © 1999 Elsevier Science Inc.

Propionic acid Propionic acidemia Behavior Neuromotor development Open field Shuttle avoidance task

PROPIONIC acidemia is an inherited disorder of organic acid metabolism caused by a deficiency of propionyl-CoA carboxylase activity (13,15). The blockage of the metabolic pathway gives rise to an increased amount of propionyl-CoA, which is spontaneously converted to propionic acid (PPA). Propionyl-CoA comes from the catabolism of the amino acids the threonine, methionine, valine, and isoleucine, as well as from odd-chain fatty acids, cholesterol, thymine, and uracil. The disorder is, therefore, characterized by the accumulation of primarily PPA and secondarily of other metabolites in tis-

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sues of affected individuals. This usually results in a severe clinical disorder characterized by dehydration, refusal to eat, vomiting, lethargy, and coma during the first days of life. Older children usually present recurrent attacks of ketoacidosis, respiratory distress, and developmental retardation. Among the frequently observed neurologic signs, axial hypotonia, extrapyramidal manifestations, focal and general seizures, and EEG abnormalities have been the most prominent. Brain structural abnormalities are frequently found. The neuropathologic findings commonly detected include delayed myelination and hypodensity of the globi pallidi (3,4,13,15). Laboratory findings include metabolic acidosis, ketonemia/ketonuria, hypoglycemia, neutropenia, and thrombocytopenia (13,15). Propionic acidemia was recently included in the group of disorders called "cerebral organic acidemias," because of the high amounts of propionic acid encountered in the brain, suggesting that the metabolite may be produced in nervous cells (14). Very little is known about the neurochemical and behavioral aspects of this disease, and suitable animal models for studying these aspects are still lacking in the literature. The objective of the present study was to develop chemically induced propionic acidemia and to investigate the effect of sustained high serum concentrations of PPA on rat development and behavior. It was not the purpose of this investigation to mimic genetic propionic acidemia, because other metabolites accumulate in the human condition.

METHOD

A total of 272 (34 litters) Wistar rats from our stock were used. Pregnant rats were housed in individual cages and left undisturbed throughout gestation. Twenty-four hours after delivery the litters were culled to eight pups, consisting of only males. Half of them were assigned to the experimental condition and the other half served as controls. The rats were weaned at 21 days. All animals had free access to commercial chow and water, and were kept on a 12 h light/dark cycle at 24 ± 1°C.

Saline-buffered PPA was administered subcutaneously from the 6th to the 28th day of life to induce propionic acidemia. Doses were determined by measuring pharmacokinetic parameters, such as apparent volume of distribution (V_d), plasma half-time ($t1/2$), and plasma clearance (CL_p) after drug administration (2). Using these parameters, doses were calculated to achieve plasma levels similar to those described in children affected by propionic acidemia. Control rats received saline solution in the same volumes. Because of the high plasma clearance, propionate was administered twice a day with an interval of 8 h between injections.

For the pharmacokinetic studies, a total of 12 litters of 8, 15, and 22-day-old rats were used. Four rats from the same litter were injected with one of two PPA doses: 1.0 or 2.0 mmol/g body weight. Blood was collected into heparinized tubes by cardiac puncture at 30, 60, 90, or 120 min after the injection. Plasma was separated by centrifugation, and PPA was determined in plasma. The semilogarithmic plot of plasma PPA concentration vs. time indicated that the drug was eliminated from a single compartment by a first-order process. The means of four PPA measurements in the plasma were used for determination of the pharmacokinetic parameters (Table 1). From these parameters, the following doses were calculated to achieve 1.0 to 5.0 mM plasma PPA concentration in PPA-treated rats of various ages: 6 to 12 days of life, 0.72 μmol/g body weight; 13 to 19 days, 1.68 μmol/g body weight; 20 to 28 days, 1.92 μmol/g body weight.

For the determination of PPA levels in plasma and brain, six litters of 8-, 15-, and 22-day-old rats were injected with the calculated doses. These animals were sacrificed by decapitation. Blood was collected into heparinized tubes and plasma was separated by centrifugation. The brain was rapidly removed and the medulla, pons, and olfactory lobes were discarded. The rest of the brain (cerebrum) and the cerebellum were weighed. The cerebrum was homogenized in saline.

Chronically treated rats of 28, 35, and 60 days of age were also sacrificed by decapitation, and had their brain dissected. Body, cerebrum, and cerebellum weights were measured.

Propionate was determined in the cerebrum homogenate and in plasma by gas chromatography according to Buchanan and Thoene (7). Briefly, an SP-1200/1% H_3PO_4 (Supelco) chromatography column was used in all analyses. Gas chromatograph conditions were as follows: oven temperature was isothermal at 150°C, carrier gas was nitrogen at a flow rate of 25 ml/min, internal standard was pivalic acid, and an FID detector was used. Body, cerebrum, and cerebellum weights were analyzed by the Student's *t*-test for unpaired samples.

Physical Development

Eighty male rats (10 litters) were used for all neurobehavioral development studies. Maturation of physical characteristics was determined daily at the appropriate ages by one experimenter that was not aware of the subject condition. Litters were inspected between 1200 and 1500 h, and progress of the same individuals from each litter was followed throughout the experiment. The date of appearance of hair, eruption of upper incisors, and eye opening was recorded using previously reported criteria (8,16,19) described in detail by Smart and Dobbing (19). Physical development data were analyzed by group (PPA or saline) × litters ANOVA.

Early Behavioral Parameters

On postnatal day 14, animal ability to turn in midair to land on all fours after being dropped back downwards from 35 cm onto a cotton wool pad (free-fall righting) was measured. Each animal was tested in three consecutive trials spaced 15 s apart, and scored one point if it landed ventrally, with all legs distant from the body in each trial (8). Therefore, a maximum of three points could be assigned to each animal. Free-fall righting scores were analyzed by group (PPA or saline) × litter ANOVA.

On postnatal day 21, animals were tested for spontaneous alternation. The apparatus was a T-maze (base, 35 cm and each arm 25 cm long). Latency to enter one arm of the maze

TABLE 1

PHARMACOKINETIC PARAMETERS OF PROPIONIC ACID (PPA) AND DOSES ADMINISTERED ACCORDING TO RAT AGE

Age (Days)	t (1/2) (min)	Vd (ml/g)	Clp (μl/min·g)	Propionate Doses (μmol/g)
8	57.0	0.07	2.95	0.72
15	25.5	0.15	4.18	1.68
22	18.0	0.68	8.33	1.92

Values are reported as the mean for four animals of each age. PPA doses were claculated from pharmacokinetic parameters to achieve plasma PPA levels of 1.0–5.0 mmol/l, t(1/2), plasma half-time; Vd, apparent volume of distribution; Clp, plasma clearance.

and arm entered were recorded. The animals were tested in consecutive trials (intertrial interval, 20 s) until they entered the contralateral arm, with a maximum of five trials. When the latency to enter one of the sides was longer than 300 s, the animal was excluded from subsequent testing (8). Latency to enter the first arm and the number of alternate trials were calculated and compared between groups by two-way ANOVA.

Adult Behavioral Parameters

After chronic administration of saline and propionate was completed, a subset of male animals was left undisturbed in their cages up to 60 days of age. These animals were consecutively subjected to a nonaversive task (open-field habituation) and to an aversive task (two-way shuttle avoidance).

Open field. The rats' ability to habituate to a new environment was assessed by subjecting the animals to two consecutive sessions in an open field, spaced 24 h apart. The apparatus consisted of a wooden box measuring 60 × 40 × 50 cm with a glass front wall, whose floor was divided by white lines into 12 equal squares. The animals were gently placed facing the rear left corner of the arena and observed for 2 min. The latency to leave the first square, number of squares crossed with the four paws, number of rearing responses, and number of fecal boli were manually recorded by an observer who was not aware of the subject's condition (21).

Shuttle avoidance. Forty-eight hours after the last open field session, the rats were subjected to two consecutive sessions spaced 24 h apart in a 50 × 25 × 25-cm shuttle-avoidance apparatus. The floor was a grid of 2-mm bronze bars spaced 10 mm apart through which 0.5-mA scrambled footshocks could be delivered. The midline of the grid was marked by a strip of acrylic, 0.5 cm wide, between the two central bars. The bars on each side of the strip were independently connected to an electric stimulator (Albarsh, Porto Alegre, Brazil). The walls were opaque, except for the front wall. After 5 min of adaptation to the box, each rat received conditioned stimuli (CS) consisting of 20 tones (80 dB) at randomly selected intervals of 10–50 s. Each CS lasted 5 s, and was immediately followed by a 0.5-mA footshock (unconditioned stimulus, US), unless the rat made a shuttle response to the tone, in which case the tone was interruped and the shock scheduled for that trial was cancelled. Twenty-four hours after the first session, rats were subjected to an identical second session. Thus, during one session, two shuttle responses could occur (i.e., the rat crossed the midline of the box): during a tone presentation (avoidance response) or during a shock delivery (escape response). Both the number of avoidance responses and the latency to escape the footshock (escape latency) were recorded automatically. The behavioral procedures were carried out between 1200–1500 h. Data from adult behavioral task were analyzed by two-way ANOVA (treatment × sessions), with the session factor treated as a within-subject factor (20).

TABLE 2

PROPIONATE CONCENTRATIONS IN CEREBRAL CORTEX OF RATS INJECTED WITH BUFFERED PROPIONIC ACID (PPA)

Age (Days)	30 Min	60 Min	90 Min	120 Min
8	0.34 ± 0.05	1.25 ± 0.26	1.13 ± 0.18	0.34 ± 0.08
15	1.0 ± 0.20	0.91 ± 0.15	0.47 ± 0.09	ND
22	0.74 ± 0.21	1.33 ± 0.18	ND	ND

PPA concentrations are expressed as μmol/g. Values are reported as mean for four animals at each age. ND—not detectable. The columns referring to minutes correspond to time after subcutaneous injection of PPA.

TABLE 3

EFFECT OF POSTNATAL CHRONIC PROPIONIC ACID (PPA) ADMINISTRATION ON BODY, CEREBRUM, AND CEREBELLUM WEIGHT OF RATS

Treatment	Saline	Propionate
	28-Day-Old	
Body (g)	65 ± 6.6	62 ± 10.3
Cerebrum (mg)	652 ± 55.6	665 ± 46.7
Cerebellum (mg)	197 ± 15.1	181 ± 22.5
	35-Day-Old	
Body (g)	85 ± 7.16	84 ± 6.1
Cerebrum (mg)	734 ± 37.5	699 ± 50.5
Cerebellum (mg)	201 ± 32.0	209 ± 16.5
	60-Day-Old	
Body (g)	159 ± 22.7	154 ± 34.0
Cerebrum (mg)	735 ± 104.7	782 ± 84.0
Cerebellum (mg)	275 ± 21.5	234 ± 39.0

Rats received PPA subcutaneously from day 6 to day 28 of life. The doses varied according to age to obtain plasma PPA levels of 1–5 mmol/l. Data are expressed as mean ± SD for 12 rats per gorup. There were no significant differences between PPA-and saline-treated rats.

RESULTS

The pharmacokinetic parameters of propionate and the doses calculated to achieve plasma PPA levels of 1.0 to 5.0 mM at the different ages are displayed in Table 1. Cerebral PPA concentration in the rats receiving buffered propionate reached a maximum between 60 and 90 min after injection (Table 2).

Body, cerebrum, and cerebellum weights of rats submitted to chronic administration of PPA did not differ from those of control rats (Table 3).

The effect of chronic PPA administration on the date of appearance of certain physical landmarks and reflexes was studied. Regarding physical features, the statistical analyses [treatment (saline or PPA) × litters [10] ANOVA] revealed that PPA-treated animals exhibited a delay in the date of appearance of coat; control: 7.4 ± 0.08 vs. PPA: 7.6 ± 0.13, $F(1,$

TABLE 4

EFFECT OF POSTNATAL CHRONIC PROPIONIC ACID (PPA) ADMINISTRATION ON DEVELOPMENT OF CERTAIN REFLEXES IN RATS

	Saline	Propionate
Free-fall righting score	2.4 ± 0.12	1.8 ± 0.14*
Spontaneous alternation		
Trials to alternate	1.2 ± 0.07	1.5 ± 0.11
Latency in first trial	14.8 ± 2.76	12.0 ± 2.11

Data are expressed as means ± SD for 40 rats per group. Free-fall righting reflex was evaluated on day 14 after birth and spontaneous alternation on day 21.

* Significantly different from control group at $p < 0.05$.

TABLE 5

EFFECT OF POSTNATAL CHRONIC PROPIONIC ACID (PPA) ADMINISTRATION ON OPEN-FIELD BEHAVIOR OF ADULT RATS

	Saline (26)		Propionate (27)	
Treatment	1st Session	2nd Session	1st Session	2nd Session
Latency to leave the first square (s)	4.0 ± 0.77	2.4 ±0.46*	3.7 ± 0.76	2.9 ± 0.55*
Number of rearings	16.1 ± 2.73	13.7 ± 2.63*	15.6 ± 3.00	16.1 ± 3.10
Number of crossing	39.1 ± 7.53	40.4 ±7.79	39.7 ± 7.6	37.7 ± 2.7
Number of fecal boli	1.7 ± 0.33	1.3 ± 0.24	1.6 ± 0.30	1.0 ± 0.2

Data are reported as means ± SEM. The number of animals in each group is given in parentheses. Rats received PPA subcutaneously from day 5 to day 28 of life. The dose schedule was varied to obtain plasma levels of 1–5 mmol/l (see text of Table 1). The task was applied on days 61 and 62 after birth.

* Statistically significant compared to the first session for the same group at $p < 0.05$.

79) = 6.84, $p < 0.05$, and a delay in eye opening; control: 12.9 ± 0.11 vs. PPA: 13.2 ± 0.17, $F(1, 79) = 4.59$; $p < 0.05$. Conversely, propionate-treated animals presented earlier upper incisor eruption; control: 9.7 ± 0.14 vs. PPA: 9.3 ± 0.17, $F(1, 79) = 7.83$; $p < 0.01$. These data indicate that chronic propionate administration causes retardation in the date of appearance of some physical parameters and advancement of others.

Regarding the reflexes, the statistical analyses [treatment (saline or PPA) × litters [10] ANOVA] revealed only a significant effect of PPA in the free-fall righting task, $F(1, 79) = 10.10$; $p < 0.01$. No effect of the organic acid on the spontaneous alternation test was observed (Table 4).

When they reached adulthood, animals were subjected to the open-field task. Statistical analyses [treatment (saline or PPA) × litters [7] × sessions [2] ANOVA, with the last factor treated as within-subject factor] of latency to leave the first square showed only a significant main effect of sessions, $F(1, 39) = 26.41$; $p < 0.001$, indicating that both groups reduced latency to leave the first square over subsequent sessions (Table 5).

On the other hand, statistical analysis of rearing scores revealed a significant treatment (saline or PPA) × session interaction because saline-treated, but not PPA-treated animals reduced their rearing scores along the sessions, $F(1, 39) = 4.32$, $p < 0.05$. This result suggests that PPA-treated animals showed a deficit in habituation to a novel environment.

The analysis of motor activity (number of crossings) of the 60-day-old rats submitted to the open-field task showed no significant effect of PPA administration on the number of crossing responses compared to saline-treated animals (Table 5). Similarly, defecation of rats submitted to the open field was similar in both groups (Table 5), suggesting that PPA does not affect emotionality.

Table 6 shows the number of shuttle-avoidance responses, intertrial crossing responses, as well as the footshock escape latencies of rats submitted to the shuttle avoidance test. Chronic propionate administration had no effect on motor activity of rats, as shown by the similar intertrial crossing scores of saline- and PPA-treated rats in both sessions, $F(1, 39) = 0.19$; $p > 0.66$. In addition, both groups presented similar footshock escape latencies along the sessions, $F(1, 39) = 9.37$; $p < 0.01$. On the other hand, analyses of the number of avoidance responses revealed that only saline-treated animals increased the number of avoidance responses along the sessions (significant treatment (saline or PPA) × sessions interaction, $F(1, 39) = 4.42$; $p < 0.05$. These findings suggest that PPA-treated animals present a deficit in the performance of the shuttle-avoidance task.

DISCUSSION

In the present study we produced high sustained levels of propionate in the blood and brains of young rats. Serum propionate levels achieved in our model were 1–5 mmol/l, similar to those found in human propionic acidemia (13,15). Brain PPA concentrations were around 1 μmol/g, and this result cannot be compared to others because there are no avaliable data in the literature. However, because the defect is appar-

TABLE 6

EFFECT OF POSTNATAL CHRONIC PROPIONIC ACID (PPA) ADMINISTRATION ON SHUTTLE AVOIDANCE BEHAVIOR OF ADULT RATS

	Saline (26)		Propionate (27)	
Treatment	1st Session	2nd Session	1st Session	2nd Session
Avoidance response (number)	7.1 ± 0.65	10.5 ± 0.74*	8.2 ± 0.68	9.1 ± 0.85
Intertrial Crossing (number)	11.8 ± 2.12	12.2 ± 1.73	13.9 ± 2.78	11.5 ± 1.93
Escape latency during the first 10 tones (s)	1.9 ± 0.33	1.1 ± 0.20	1.7 ± 0.31	1.3 ± 0.25

Data are reported as means ± SEM. The number of animals in each group is given in parentheses. Rats received PPA subcutaneously from day 5 to day 28 of life. The dose schedule was varied to obtain plasma levels of 1–5 mmol/l (see text or Table 1). The task was applied on days 64 and 65 after birth. Animals were submitted to 20 trails using a 0.5 mA footshock.

* Statistically significant compared to the first session for the same group at $p < 0.05$.

ently expressed in brain, patients with propionic acidemia may have brain PPA concentrations higher that 1 μmol/g (14). The drug was administered during a period characterized by intense cellular proliferation and synaptogenesis in the various cerebral structures involved in learning/memory in rats (11,12,18,22). Our results about the pharmacokinetic parameters and the permeability of the blood–brain barrier to propionate revealed that they change with age. Although the pharmacokinetic parameters indicated an increase in propionate metabolization and excretion with advancing age, propionate permeability through the blood–brain barrier decreased with age. It was seen that the blood–brain barrier of developing rats (28-day-old rats) is less permeable to exogenous propionate, a fact that emphasizes the importance of studying the effect of chronic peripherally administered propionate on the central nervous system (CNS) in early postnatal life.

We investigated the effect of chronic postnatal administration of propionate on rat neurobehavioral development. Neuromotor development was checked from the time of PPA administration to the end of treatment, whereas the behavioral studies were performed 7 weeks after treatment (60-day-old rats). By doing so, we aimed to investigate a possible persistent effect of propionate on certain behavioral tasks, because behavioral studies performed during or soon after treatment may be difficult to interpret (1).

Chronic propionate administration had no effect on body, cerebrum, or cerebellum weight. These results suggest that propionic acid injection does not cause undernutrition in the animals. Therefore, this common undesired variable in behavioral studies can be ruled out as a cause of the behavioral alterations observed (9,19).

Chronic propionate administration caused a delay in the date of appearance of some physical features and the free-fall righting reflex. Propionate treatment also caused long-lasting behavioral alterations, as judged by the altered performance of PPA-treated animals in the open-field and two-way shuttle avoidance tasks. In the open-field task we observed that PPA-treated animals, in contrast to control rats, did not present significant differences in rearing responses between sessions. Because habituation to a novel environment, such as the open-field box, is usually associated with a decrease in the number of rearing responses in the second session compared to the first (20,21), we presume that PPA-injected rats did not habituate to the open-field task. On the other hand, anxiety-related responses (defecation and latency to leave the first square) were not affected by propionate treatment in this task, indicating that PPA did not interfere with emotionality. Motor activity assessed by the number of crossings was also not affected by PPA treatment, suggesting no effect of the metabolite on this parameter.

In the shuttle-avoidance task, PPA-treated rats did not increase the number of footshock escape responses in the second session, a fact that may be interpreted as a learning deficit. Motor activity, measured by the intertrial crossing, was not affected by the metabolite in the same task, suggesting that the effects observed in the footshock escape responses were not related to motor impairment in these animals.

Thus, we found in the present study that chronic administration of propionate to developing rats causes long-lasting behavioral changes related to both aversive memory and habituation to novelty. This is particularly interesting in view of the psychomotor delay/mental retardation characteristic of propionic acidemic children. However, the significance of these findings and their possible relationship to the human condition is far from clear. We presume that the brain propionate concentrations achieved in our chemical model of propionic acidemia (1 μmol/g) may be sufficient to cause metabolic alterations in pathways involved in learning/memory and in other neurological functions. At this point, it should be stressed that patients with propionic acidemia may have brain propionate concentrations higher than 1 μmol/g (14).

The underlying neurochemical mechanisms responsible for the neurobehavioral developmental delay in rats due to propionate treatment and of propionic acidemic children remain obscure. It is tempting to speculate that this delay may be related to the findings observed in various recent in vitro studies carried out in our laboratory demonstrating that propionic acid impairs in vitro brain lipid biosynthesis (17) and energy production (5), and decreases *N*-acetylneuraminic acid content in cerebellum (6), and the phosphorylation of cytoskeletal proteins in the cerebral cortex of rats (10) after chronic administration.

In conclusion, we believe that the presently described chemically induced animal model of propionic acidemia may be of value in elucidating some aspects of the neurological dysfunction occurring in human propionic acidemia.

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REFERENCES

1. Andersen, A. E.; Guroff, G.: Enduring behavioral changes in rats with experimental phenylketonuria. Proc. Natl. Acad. Sci. USA 69:863–867; 1972.
2. Benet, L. Z.; Schemer, L. B.: Pharmacokinetics: The dynamics of drug absorption, distribution and elimination. In: Gilman, A. G.; Goodman, L. S.; Rall, T. W.; Murad, F.; eds. The pharmacological basis of therapeutics. New York: Macmillan Publishing Company; 1987: 3–34.
3. Bergman, J. W.; Van Der Kanaap, M. S.; Smeitink, J. A. M.; Duran, M.; Dorland, L.; Valk, J.; Poll, B. T.: Magnetic resonance imaging and spectroscopy of the brain in propionic acidemia: Clinical and biochemical considerations. Pediatr. Res. 3:404–409; 1996.
4. Brismar, J.; Ozand, P. T.: CT and MR of the brain in the diagnosis of organic acidaemias. Experiences from 107 patients. Brain Dev. 16:104–124; 1994.
5. Brusque, A. M.; Malfussi, H. F. C.; Rocha, M. O.; Dutra-Filho, C. S.; Wannmacher, G. M. D.; Wajner, M.: Propionic acid inhibits in vitro CO_2 production in cerebellum and cerebral cortex of suckling rats. Med. Sci. Res. 25:347–349; 1997.
6. Brusque, A. M.; Terracciano, S. T.; Fontella, F. U.; Vargas, C. R.; Da Silva, C. G.; Wyse, A. T. S.; Trindade, V. M. T.; Wannmacher, C. M. D.; Wajner, M.: Chronic administration of propionic acid reduces ganglioside *N*-acetylneuraminic acid (G-NeuAc) concentration in cerebellum of young rats. J. Neurol. Sci. 158:121–124; 1998.
7. Buchanan, D. N.; Thoene, J. G.: Volatile organic acid profiling in physiological fluids using gas chromatography/mass spectrometry, In: Hommes, F. A., ed. Techniques in diagnostic human biochemical genetics: A laboratory manual, 1st ed. New York: John Wiley & Sons, Inc.; 1991:133–142.
8. Da Silva V. A.; Malheiros, L. R.; Bueno, F. M. R.; Effect of tolu-

ene exposure during gestation on neurobehavioral development of rats and hamsters. Braz. J. Med. Biol. Res. 23:533–537; 1990.
9. Davis, P. H.; Squire, L. R.: Protein synthesis and memory: A review. Psychol. Bull. 96:518–559; 1984.
10. De Mattos-Dutra, A.; Sampaio de Freitas, M.; Dourado Poli, D.; Pessoa-Pureur, R.; Wajner, M.: Effects of methylmalonic and propionic acids on the in vitro incorporation of 32P into cytoskeletal proteins from the cerebral cortex of young rats. Neurochem. Int. 33:75–82; 1998.
11. Dreyfus, H.; Ferret, B.; Harth, S.; Giorgio, A.; Durand, M.; Freysz, L.; Massarelli, R.: Metabolism and function of gangliosides in developing neurons. J. Neurosci. Res. 12:311–322; 1984.
12. Enesco, M.; Leblond, C. P.: lncrease in cell number as a factor in the growth of organs and tissues of the young male rat. J. Embryol. Exp. Morphol. 10:530–562; 1962.
13. Fenton, W. A.; Rosemberg, I. E.: Disorder of propionate and methylmalonate metabolism ln: Scriver, C. R.; Beaudet, A.; Sly, W. S., eds. The metabolic and molecular bases of inherited diseases; Valle disease, 7th ed. New York: McGraw-Hill; 1995:1423–1449.
14. Hoffmann, G. F.; Meier-Augenstein, W.; Stocker, S.; Surtees, R.; Rating, D.; Nyhan, W. L.: Physiology and pathophysiology of organic acids in cerebrospinal fluid. J. lnheret. Metab. Dis. 16:648–669; 1993.
15. Lehnert, W.; Sperl, W.; Suormala, T.; Baumgartiner, C. R.: Propionic acidaemia: Clinical, biochemical and therapeutic aspects. Experience in 30 patients. Eur. J. Pediatr. 153 (Suppl. 1): 68–80; 1994.
16. Mello, C. F.; Somer, J. E.; Tavoaroni, V.; Graciolli, R.; Wu, V.; Torres, G.; Aguiar, F.; Wajner, M.; Wannmacher, C. M. D.: Effects of postnatal methylmalonate administration on neurobehavior development of rats. Braz. J. Med. Biol. Res. 27:655–661; 1994.
17. Mello, C. F.; Rubin, M.; Coelho, J. C.; Wajner, M.; Souza, D. O. G.: Effect of methylmalonate and propionate on [^{3}H] glutamate binding, adenylcyclase activity and lipid biosynthesis in cerebral cortex of rats. Biochem. Mol. Biol. Int. 42:1143–1150; 1997.
18. Roisen, F. J.; Bartfeld, H.; Nagele, R.; Yorke, G.: Gangliosides stimulation of axonal sprouting in vivo. Science 214:577–578; 1981.
19. Smart, J. L.; Dobbing, J.: Vulnerability of developing brain II. Effect of early nutritional deprivation on reflex ontogeny and development of behavior in the rat. Brain Res 28:85–95; 1971.
20. Walden, A. M.: Studies of exploratory behaviour in the albino rat. Psychol. Rep. 22:483–493; 1968.
21. Walsh, R. N.; Cummins, R. A.: The open-field test: A critical review. Psychol. Bull. 83:482–504; 1976.
22. Winick, M.; Noble, A.: Quantitative changes in DNA, RNA and protein during prenatal and postnatal growth in rat. Dev. Biol. 12:451–466; 1965.