Original Article

Neurobehavioral Assessment of Rats Exposed to Yttrium Nitrate during Development*

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Abstract

Objective The aim of this study was to assess the effects of yttrium nitrate on neurobehavioral development in Sprague-Dawley rats.

Methods Dams were orally exposed to 0, 5, 15, or 45 mg/kg daily of yttrium nitrate from gestation day (GD) 6 to postnatal day (PND) 21. Body weight and food consumption were monitored weekly. Neurobehavior was assessed by developmental landmarks and reflexes, motor activity, hot plate, Rota-rod and cognitive tests. Additionally, brain weights were measured on PND 21 and 70.

Results No significant difference was noted among all groups for maternal body weight and food consumption. All yttrium-exposed offspring showed an increase in body weight on PND 21; however, no significant difference in body weight for exposed pups versus controls was observed 2 weeks or more after the yttrium solution was discontinued. The groups given 5 mg/kg daily decreased significantly in the duration of female forelime grip strength and ambulation on PND 13. There was no significant difference between yttrium-exposed offspring and controls with respect to other behavioral ontogeny parameters and postnatal behavioral test results.

Conclusion Exposure of rats to yttrium nitrate in concentrations up to 45 mg/kg daily had no adverse effects on their neurobehavioral development.

Key words: Rare earth elements; Yttrium; Developmental neurotoxicity; Neurobehavior

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INTRODUCTION

Rare earth elements (REEs) consist of 17 transition metals in Group III of the Periodic Table. Their widespread use in agriculture and industry has resulted in increasing amounts of REEs being released into the environment, thereby increasing the risk of its accumulation in humans. Studies of the biological effects of REEs and their underlying mechanisms of action have attracted considerable attention. Thus far, it is known that REEs, like many other heavy metals, affect tissues of the lung, liver, kidney, spleen, and other organs[1]. REEs may also affect brain development in animals and human beings. Briner and colleagues believed that lanthanum (La) has teratogenic effects on behavior that include subtle but detectable effects on mouse development[2]. Feng and colleagues found that subchronic exposure of rats to La can alter the concentration of DNA and the ratio of protein to DNA in the brain, thereby significantly impairing

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memory and learning abilities[3]. He and colleagues came to a similar conclusion based on their finding of changes in homeostasis for Ca^{2+}/Ca^{2+}-ATPase and in the inhibitory activity of certain antioxidant enzymes[4]. An epidemiological investigation by Zhu and colleagues revealed that children living in REE-rich regions (especially regions rich in heavy REEs) had lower intelligence quotients (IQ) and worse memory than those living in areas with normal REE levels. This suggests that long-term exposure to REEs can affect brain function[5].

Developmental neurotoxicity studies are developed to collect data on the functional disturbances and morphological hazards to the nervous system arising in the offspring from exposure of the mother during pregnancy and lactation, and to provide dose-response characterizations of these exposures[6]. Because of the limitations involved in carrying out developmental neurotoxicity studies in humans, there is a strong need for animal experiments to gather such data[7]. For example, rat pups randomly chosen from among control and exposed litters could be tested for gross neurological and behavioral abnormalities using assessments of physical development, behavioral ontogeny, motor activity, motor and sensory function, learning, and memory. Additionally, brain weight and neuropathology could be monitored throughout postnatal development and adulthood[8].

It has been reported that human exposure to compounds containing yttrium, a heavy REE widely used in industry and medical, may cause lung disease. Experiments in rats and hamsters also have shown that this silvery-metallic transition metal, which is chemically related to the lanthanides, can translocate to the skeleton and liver[1]. However, studies of the developmental neurotoxicity of yttrium are lacking, no official no observed adverse effect level (NOAEL) or acceptable daily intake (ADI) value for yttrium either. In the present study, we investigated the effects of yttrium on the postnatal development of rat pups, with a particular interest in neurobehavioral development, which was evaluated using current international test guidelines[8]. Our results could provide specific scientific data for the food safety risk assessment of yttrium or rare earth elements.

MATERIALS AND METHODS

Test Substance

A yttrium nitrate $Y(NO_3)_3$ solution was prepared by dissolving yttrium oxide (purity >99.99%; Beijing Research Institute of Nonferrous Metals, Beijing, China) in nitric acid and diluting it to a pH of 6 using 1 mol/L sodium hydroxide and deionized water.

Animals and Method of Administration

Sexually mature virgin female and male Sprague-Dawley (SD) rats were obtained from the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China). After 2 weeks of acclimatization, each female rat was mated with a resident male rat of the same strain. Evidence of a vaginal plug was used to indicate successful mating and Day 0 of pregnancy. Throughout the experiment, the animals were kept in a room with controlled illumination (a 12-h light/dark cycle), temperature (20-25 °C), and humidity (40%-70%) and were fed regular rat chow and water *ad libitum.* The procedures were carried out according to ethics guidelines for the care and use of animals in research formulated by the China National Center for Food Safety Risk Assessment.

Mated female rats were randomly divided into four groups of 20 animals each. From gestation day (GD) 6 to postnatal day (PND) 21, the pregnant rats were housed singly and received one of four doses (0, 5, 15, or 45 mg/kg) of the $Y(NO_3)_3$ solution by gavage each day. Mean maternal weight and food consumption were calculated for each group on GD 0, 3, 7, 10, 14, 17, and 21 and on PND 1, 4, 7, 10, 14, 17, and 21. Pregnant females were allowed to deliver their pups spontaneously and nurse them. On PND21, the dams were sacrificed and subjected to a gross examination. Pregnancy status was determined for those rats that failed to deliver.

Each litter was observed daily for signs of toxicity. Body weight was recorded on PND 1, 4, 7, 10, 14, 17, and 21 and weekly thereafter until PND 70. Food consumption was calculated weekly from PND 21 to PND 70. On PND 1, the number of live and dead pups was determined and the sex ratio of the pups was recorded. On PND 4, the number of pups in each litter was reduced to 8 (4 males and 4 females). The pattern of pup assignment, which appears in Table 1, is based on OECD 426 guidelines, with slight modifications.

The first set of pups (20 per sex and dose level; 1 male and 1 female per litter) was evaluated for pre-weaning behavior using behavioral ontogeny tests and for histopathology. Following weaning on PND 21, half of the pups (10 per sex and dose level)

were sacrificed and their brains were removed, weighed, and frozen for further research. The other half of the pups (*n*=10) were anesthetized using pentobarbital sodium and perfused with 4% paraformaldehyde. Their brains were then removed and weighed.

The second set of pups was evaluated for locomotor activity (on PND 13, 17, 21, and 67) and subjected to the rotating rod test (PND 25) and the hot plate test (PND 24 and 61). Sexual maturation was also determined. From this group, 10 pups per gender and dose level were anesthetized and fixed using a perfusion of 4% paraformaldehyde on PND 70. Their brains were then removed, weighed, and processed for neuropathological examination.

A third set of pups was subjected to the Morris water maze test. Half of them were tested on PND 25 and the other half on PND 62. On PND 70, 10 males and 10 females were sacrificed. Their brains were also removed and weighed.

The fourth set of pups was for another experiment. Half of them were exposed to $Y(NO_3)_3$ by gavage from PND 21 until PND 70. The remaining pups were sacrificed.

Postnatal Development of Offspring

Postnatal neurobehavioral development was evaluated beginning PND 4 by observing the surface-righting reflex on PND 4, cliff avoidance on PND 6, forelimb grip strength on PND 12, startle response on PND 13, homing reflex on PND 13, and air-righting reflex on PND 17, testes descent on PND 27, and vaginal opening on PND 31.

Locomotor Activity

Locomotor activity was assessed on PND 13, 17, 21, and 67 using the JLBehv animal locomotion analysis system (Jiling Software Technology Co, Ltd; Shanghai, China). Each rat was tested for 6 min in a plastic box surrounded by black plastic enclosures to decrease the risk of distractions in the external environment. Data for total locomotor activity, average speed, ambulatory time, and rest time were tabulated.

Morris Water Maze Test

To assess hippocampus-dependent spatial learning and memory, pups were tested in the Morris water maze on PND 25 or PND 62 as described previously[9], with slight modifications. Briefly, a black circular pool (150 cm in diameter and 50 cm in height) filled with water (23 °C) was placed in the experimental room with fixed visual clues on walls. The pool was divided into four quadrants, and an escape black platform (diameter: 11 cm) was placed 1 cm below the water surface in the middle of one of the quadrants. The position of the platform was not changed throughout the experiment. Rats were trained in three trials daily over 5 consecutive days. In each trial, the rats were placed in the water facing the wall at one of the three starting positions (not the platform quadrant) and were permitted to swim until they reached the platform. If a rat failed to find the platform within 60 s, it was guided manually toward the platform by the researcher for 15 s. Each trial was recorded using a video tracking

Table 1. Assignment of Pups to Each Test

No of Pups[a]		Pups Assigned to Each Test	Test
Male	Female		
1	5	20m + 20f	Behavioral ontogeny
		10m + 10f	Brain weight and neuropathology evaluation (PND 21)
		10m + 10f	Brain weight (PND21)
2	6	20m + 20f	Detailed clinical observations
		20m + 20f	Motor activity
		20m + 20f	Sexual maturation
		20m + 20f	Motor and sensory function
		10m + 10f	Young adult brain weight and neuropathology evaluation (PND70)
3	7	10m + 10f[b]	Learning and memory (PND 25)
		10m + 10f[b]	Learning and memory on PND 62 and brain weight on PND 70
4	8	10m + 10f	For further research: daily exposure to $Y(NO_3)_3$ through PND 70
		10m + 10f	Sacrificed

Note. [a]Male pups are numbered 1 through 4; female pups are numbered 5 through 8. [b]Different pups are used for twice cognitive tests.

system; the escape latency and distance were measured using SMG-2 software (Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College). For the probe trial, the hidden platform was removed. The animal was then released at the same starting position and allowed to swim freely for 60 s. The time and distance crossed in the target quadrant were recorded. For the visible platform task, performed on the final day of the test, the platform was placed so that it protruded 2 cm above the surface of the water.

Rota-rod Test

This test is designed to evaluate coordination and balance. It was carried out by placing a rat on a rotating drum and measuring the length of time the animal was able to maintain its balance while walking on top of a rod that sat 15 cm above the base. The rod was divided into five equal segments by six disks. The animals were trained before being tested by being placed on the rod while it rotated at a speed of 20 rpm. The length of time the rat could stay on the rod without falling was recorded. The test was repeated three times at 30-min intervals on PND 25. Each rat was allowed to stay on the rod a maximum of 300 s.

Hot Plate

This test was carried out to evaluate nociception. The rat was placed on a hot-plate apparatus that was maintained at 52 °C. The time until the rat lifted or licked a paw was recorded. The measurement was repeated three times on PND 24 and on PND 61, and the average of the three values was used as the baseline thermal nociceptive threshold.

Statistics

The Morris water maze test results were analyzed by a multivariate analysis of variance (ANOVA) of the general linear model (GLM) to determine the effects of between-subject factors (group), within-subject factors (day), and group by day of interaction on the dependent variables. Group differences on separate acquisition days, the effects on probe trial tests and the visible platform task, as well as body weight, food consumption data, and other behavioral test results were analyzed using one-way ANOVA. The significance of intergroup differences was analyzed using a two-sided Dunnett test. Significance was defined by P value <0.05[10], using SPSS 11.5 software.

RESULTS

Maternal Rats

General Observation All maternal rats survived to the scheduled necropsy. There were no clinical signs or toxic effects that could be attributed to the administration of $Y(NO_3)_3$. An evaluation of the rats that failed to deliver revealed no implantation sites, indicating a negative pregnancy status for each animal.

Body Weight and Food Consumption There is no significant difference in body weight among the animal groups during gestation and lactation (Tables 2 and 3). Mean overall food consumption was also similar among all animal during gestation and lactation (data not shown).

Table 2. Mean(±s) Change in Maternal Weight (g) during Gestation (n=20)

Group	Gestation			
	GD1	GD 7	GD 14	GD 21
Controls	256.13±13.62	302.17±15.99	348.58±21.54	447.02±24.14
5 mg/(kg·d)	255.74±24.40	304.88±23.66	347.98±29.62	447.85±41.66
15 mg/(kg·d)	257.88±19.31	303.95±20.25	345.82±24.07	451.70±31.63
45 mg/(kg·d)	256.24±15.19	302.33±14.82	346.37±16.06	453.88±26.19

Note. There were no significant differences (P>0.05).

Table 3. Mean(±s) Change in Maternal Weight (g) During Lactation (n=20)

Group	Lactation			
	PND 1	PND 7	PND 14	PND 21
Control	336.38±20.34	357.86±21.33	361.48±24.68	344.73±20.18
5 mg/(kg·d)	339.51±28.22	362.70±30.85	359.98±29.19	344.16±25.90
15 mg/(kg·d)	338.11±21.02	360.76±20.41	365.04±17.76	341.89±26.81
45 mg/(kg·d)	336.75±15.63	358.19±15.33	359.17±20.50	344.54±17.30

Note. There were no significant differences (P>0.05).

Litters

Litter data are summarized in Table 4. The postnatal survival data, the mean number of pups, the percentage of males per litter, the average pup weight, and the PND of testes descent or vaginal opening were not affected by any dosage of the yttrium solution.

Pup weights from PND 4 to PND 21 are summarized in Table 5. Both male and female groups exposed to yttrium demonstrated an increase in mean body weight compared with controls beginning PND 4. The difference in weight gain between PND 4 and PND 21 for all male rats given 5 or 15 mg/kg daily of the yttrium solution was statistically significant (P<0.05). The results for female pups were similar to those of male pups, although the difference never reached statistical significance.

Post-weaning female pup weights and weekly food consumption are displayed in Tables 6 and 7. Body weights were significantly different on PND 21 in the animals that received the 5 mg/kg or the 45 mg/kg daily dose of the yttrium solution; the same was true on PND 28 for those receiving 5 mg/kg or 15 mg/kg daily (P<0.05). Weekly food consumption increased in the 5 mg/kg daily group during the first week, whereas a relatively low intake was observed during the third and fifth week for animals receiving the 15 mg/kg daily dose of the yttrium solution. Animals that received the 45 mg/kg dose consumed a larger amount of food during the sixth and seventh week. However, these changes did not reveal a dose-response relationship and were not considered related to yttrium exposure. Moreover, no significant difference in body weight or weekly food consumption was noticed between male yttrium- exposed groups and control group during the entire post-weaning period, except for the 5 and 45 mg/kg daily groups, which had significantly higher body weights on PND 21, and the 5 mg/kg daily group, which had a higher food intake during the first week (male results not shown).

Table 4. Mean(±s) Change in Litter Parameters (n=20)

Parameter	Controls	Daily Dose		
		5 mg/kg	15 mg/kg	45 mg/kg
Survival at PND 0, % of litter	100.0±0.0	99.5±2.0	99.4±2.0	98.4±3.5
Pups per litter, n	14.40±2.52	13.40±3.14	14.60±2.62	14.45±2.28
Sex at birth, % males per litter	47.8±12.0	53.8±13.4	43.5±13.6	49.4±12.8
Average pup weight, g	6.65±0.51	7.01±0.85	6.80±0.52	6.67±0.49
Survival to PND 4, % of litter	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Survival to PND 21, % of litter	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Testes descent, PND	27.20±0.41	27.15±0.37	27.25±0.44	27.20±0.41
Vaginal opening, PND	32.95±0.51	32.75±0.64	33.05±0.51	32.90±0.45

Note. There were no significant differences (P>0.05).

Table 5. Mean(±s) Pup Weight (n=20)

Gender	Postnatal Day	Controls, Weight, g	Weight (g) per Daily Dose		
			5 mg/kg	15 mg/kg	45 mg/kg
Males	4	9.9±1.0	10.7±1.4	10.5±1.1	10.7±1.0
	7	16.9±1.8	17.8±2.0	17.9±1.8	18.1±1.1
	10	25.0±2.4	26.2±1.7	25.6±2.2	26.0±2.8
	14	34.8±3.1	36.3±2.1	35.9±2.5	36.2±2.2
	17	40.1±3.3	41.7±2.3	42.0±3.0	42.0±3.0
	21	50.2±4.5	53.6±3.7*	53.6±3.9*	53.5±4.1*
	4-21	40.3 ±3.8	42.9±2.8*	43.1±3.4*	42.8±3.5
Females	4	9.6±1.0	10.2±1.1	10.1±0.8	10.2±0.9
	7	16.2±2.0	17.2±1.1	17.2±1.4	17.3±1.0
	10	24.2±2.5	25.5±1.7	24.9±2.0	25.3±1.8
	14	34.0±3.4	35.6±2.1	34.9±1.8	34.9±1.2
	17	39.2±3.7	40.7±2.2	41.3±2.3	41.0±1.6
	21	49.7±4.9	52.7±3.7	52.9±2.4	52.2±2.4
	4-21	40.1±4.2	42.6±3.2	42.8±2.4	42.0±2.0

Note. *P<0.05 *vs.* controls.

Pre-Weaning Behavioral Tests

As shown in Table 8, no significant difference was found among the exposed groups, except in terms of the female pup forelimb grip strength, the duration of which decreased significantly in the group receiving 5 mg/kg daily. A similar trend was observed for pups receiving 15 and 45 mg/kg daily, but the differences among these groups were not significant (P>0.05).

Post-Weaning Behavioral Tests

Rota-rod and Hot Plate Test There were no significant differences among groups of pups in either gender on the Rota-rod tests and hot plate tests (data not shown).

Locomotor Activity Test Figures 1 and 2 illustrate the total distance and the ambulatory time for both male and female pups on PND 13, 17, 21, and 67. A statistically significant (P<0.05) decrease in

Table 6. Mean(±s) Female Pup Weight (n=40)

Postnatal Day	Controls, Weight, g	Weight (g) per Daily Dose		
		5 mg/kg	15 mg/kg	45 mg/kg
21	50.5±4.4	53.9±3.4*	52.6±3.3	52.7±4.9*
28	86.6±7.0	91.2±7.3*	90.3±5.7*	88.8±7.3
35	142.4±9.9	146.3±11.7	144.2±8.3	143.1±11.6
42	185.8±13.0	189.1±15.2	186.1±12.0	187.4±15.3
49	217.9±13.6	220.1±15.3	216.9±15.8	220.0±17.2
56	249.2±18.2	250.6±19.8	245.7±16.6	250.4±20.8
63	273.9±20.3	275.6±23.0	271.7±20.8	277.6±24.0
70	292.5±24.0	293.4±25.3	292.5±23.6	297.3±26.2

Note. *P<0.05 *vs*. controls.

Table 7. Mean(±s) Post-weaning Weekly Food Consumption, Females (n=40)

Week	Food Intake (g),Controls	Food Intake (g) per Daily Dose		
		5 mg/kg	15 mg/kg	45 mg/kg
1	72.1±4.3	75.6±5.8*	73.7±3.9	73.5±4.9
2	119.7±8.0	122.2±8.0	119.7±6.6	120.0±8.7
3	152.6±8.6	152.7±12.0	146.4±8.3*	151.2±9.4
4	154.5±7.3	152.8±7.6	146.9±7.4*	156.1±9.4
5	160.8±14.2	160.7±13.6	153.5±7.6*	165.2±13.7
6	167.1±10.6	167.9±14.5	162.5±7.6	176.4±16.8*
7	162.6±13.5	166.5±17.4	161.6±11.7	171.9±14.8*

Note. *P<0.05 *vs.* controls.

Table 8. Results of Pre-weaning Neurological Development Tests (mean±s, n=20)

Test	Controls	Results per Daily Dose		
		5 mg/kg	15 mg/kg	45 mg/kg
Surface righting reflex, PND	4.10±0.31	4.10±0.31	4.05±0.22	4.15±0.37
Cliff avoidance, PND	6.20±0.41	6.30±0.57	6.15±0.37	6.25±0.55
Male forelimb grip strength, s	20.64±14.23	19.16±11.21	19.75±8.77	20.10±8.50
Female fore-limb grip strength, s	26.12±15.37	17.11±11.13*	18.99±11.83	18.08±7.47
Startle response, PND	13.10±0.31	13.30±0.57	13.15±0.49	13.15±0.37
Male homing reflex, s	21.20±11.92	20.00±13.72	16.65±8.81	22.45±13.61
Female homing reflex, s	22.28±11.46	21.85±12.67	18.75±12.07	17.30±11.61
Air righting reflex, PND	17.05±0.22	17.05±0.22	17.05±0.22	17.00±0.00

Note. *P<0.05 *vs.* controls.

ambulatory time was noted in the 5 mg/kg daily group for both genders on PND 13. No other differences in either total distance or ambulatory time were found for either exposed group versus the concurrent control group.

Morris Water Maze Test There was no significant difference or trend between groups during the learning and memory trials, which started on PND 25 and PND 62, respectively. Spatial reference memory was tested on the sixth day, and no differences were observed in terms of the time spent in the quadrant in which the platform was previously located; this was assessed using the probe trial. All groups were subjected to a test of their ability to escape to a visible platform, but the difference in findings was not significant (data not shown).

Brain Endpoints

Brain weights taken on PND 21 and PND 70 are summarized in Table 9. On PND 21, all exposed groups showed an increase in body weight; however, the differences were only significant for males that received 15 or 45 mg/kg daily and females that received 5 or 15 mg/kg daily (P<0.05) compared with concurrent controls. As a result brain weight (as a percentage of body weight) was significantly lower in male rats given 15 mg/kg yttrium solution daily and female rats given 5 mg/kg (P<0.05).

No statistically significant difference in weight gain was seen in rats that had been perfused with 4% paraformaldehyde on PND 21 or PND 70, except for the 15 mg/kg male rats, which had a lower body

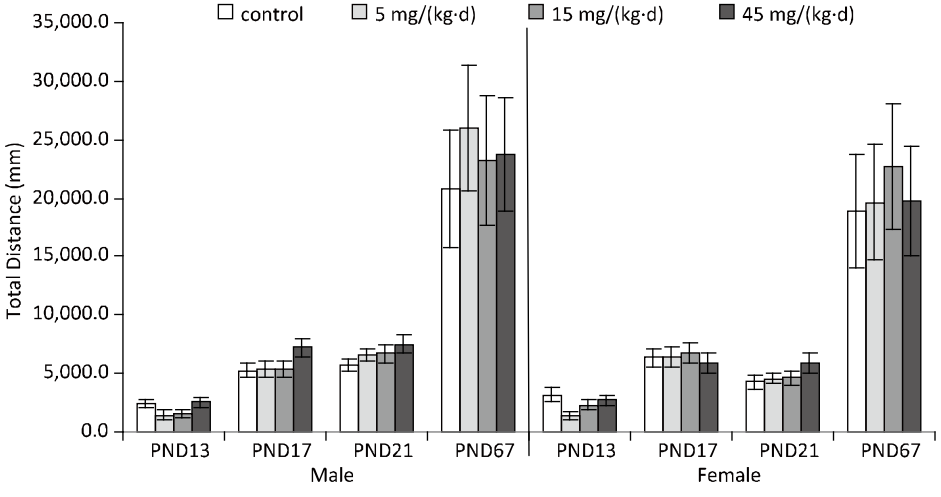

Figure 1. Total distance (mean±sx) by postnatal day for males and females.

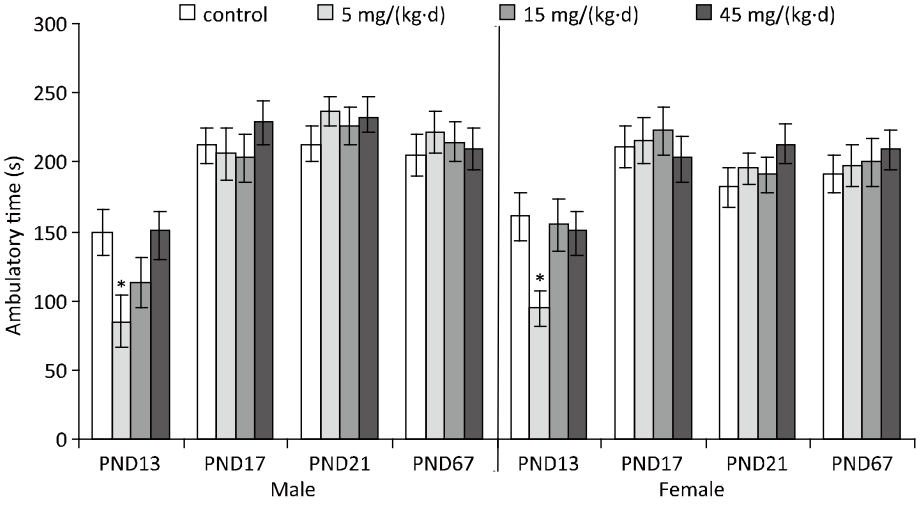

Figure 2. Ambulatory time (mean±sx) by postnatal day for males and females. *P<0.05 *vs.* controls.

weight and increased brain weight (as a percentage of body weight) on PND 70 (P<0.05). In addition, male rats that received 45 mg/kg daily demonstrated an increase in brain weight (as a percentage of body weight) on PND 70 (P<0.05; data for these variables are not shown). These changes were not considered related to maternal test administration due to the lack of a dose-response relationship.

DISCUSSION

The blood brain barrier is a highly selective permeability barrier that separates the circulating blood from the extracellular fluid in the central nervous system. Xia believed that REEs could not pass through an intact blood brain barrier or could only pass through at a very low rate of diffusion[11]. By contrast, in individuals with a developing central nervous system that has not developed an intact blood brain barrier, neurotoxins could pass through more readily, resulting in severe injury to the immature brain. Estevez and colleagues reported that REEs can accumulate in the brain of rat pup after the pregnant rat is exposed to water containing REEs[12]. He and colleagues also found that REEs administered orally can increase growth rate in rats and broilers, which indicates that even at a low dose, REEs can stimulate the release of growth hormone and affect blood levels of triiodothyronine and thyroxine, which also influence the rate of metabolism and growth[13]. In our study, $Y(NO_3)_3$ was administered to pregnant rats by gavage from GD 6 to PND 21. Their pups were, thus, exposed to yttrium both in utero and postnatally (through the mother's milk). We found that all yttrium-exposed pups gained weight at a significantly faster rate between PND 4 and PND 21 compared to non-exposed pups but that the difference in body weight between exposed groups and controls was no longer significant 2 weeks or more after the yttrium solution was discontinued. These findings suggested that yttrium may gain comparatively easy entry into the brain during the development and induce an increase in the body weight. However, it should be noted that although an increase in the rate of growth can be advantageous in some species under certain circumstances, the accumulation of REEs in the brain could affect the cell differentiation and proliferation, or disrupt neuroendocrine activity, and these effects could influence neurobehavior over a longer period[14]. Our research showed that both male and female pups receiving 5 mg/kg daily demonstrated a decrease in ambulatory time on PND 13 in the locomotor activity test and that female pups in this group demonstrated a significantly shorter duration of forelimb grip strength. These transient changes may be related to maternal test administration, but they should not be taken as toxic effects due to the lack of a dose-response relationship.

In toxicology, phenomenon of *hormesis* is characterized by low-dose stimulation and high-dose inhibition. The animals in our study exposed to yttrium both in utero and postnatally demonstrated a greater increase in body weight on PND 21, although the exposure to yttrium was quite low. But in our another experiment, of particular interest is the observation that the males receiving the greatest

Table 9. Mean(±s) Absolute and Relative Brain Weights of Pups on PND 21 and 70 (n=10)

PND	Brain Endpoints	Controls	Weights (g) per Daily Dose		
			5 mg/kg	15 mg/kg	45 mg/kg
21	Male body weight, g	54.56±3.26	58.23±5.82	61.80± 2.94*	59.68±4.09*
	Male brain weight, g	1.48±0.08	1.53±0.08	1.52±0.09	1.52±0.08
	Male brain weight, %[a]	2.72±0.20	2.65±0.24	2.47±0.15*	2.56±0.15
	Female body weight, g	51.23±5.19	59.51±5.26*	57.13±5.87*	54.53±5.02
	Female brain weight, g	1.44±0.10	1.45±0.05	1.49±0.07	1.44±0.08
	Female brain weight, %[a]	2.83±0.30	2.46±0.23*	2.62±0.23	2.65±0.26
70	Male body weight, g	449.63±38.91	442.66±28.56	454.76±45.44	438.91±43.34
	Male brain weight, g	2.10±0.07	2.11±0.09	2.09±0.12	2.19±0.11
	Male brain weight, %[a]	0.47±0.05	0.48±0.03	0.46±0.04	0.50±0.05
	Female body weight, g	281.03±35.87	287.01±28.51	275.59±15.28	286.91±33.55
	Female brain weight, g	1.99±0.09	1.98±0.06	1.99±0.08	1.99±0.10
	Female brain weight, %[a]	0.72±0.08	0.70±0.07	0.72±0.04	0.70±0.06

Note. *P<0.05 *vs.* controls. [a]Brain weight /100 g body weight.

amount of $Y(NO_3)_3$ (45 mg/kg daily) by gavage from PND 21 to PND 70 demonstrated a statistically significant decrease in body weight by the end of the experiment; those receiving 5 and 15 mg/kg daily had somewhat higher or lower body weights, respectively (data not published yet). Thus, the pups in our experiments demonstrated that yttrium had a hormesis effect on the body weight. However, the hormesis phenomenon associated with REEs is not only limited to body weight. Jiang and colleagues[15], for example, reported that ICR mice given 0.002, 0.02, 0.2, 2, and 20 mg/kg of the REE lanthanum nitrate for 4 weeks demonstrated an 'U-shaped pattern' in the water maze test, with 0.2 mg/kg daily having the shortest time; and an 'inverted U-shaped pattern' in the amount of phosphorylated CREB and JNK proteins in the hippocampus, with the highest level in 0.2 mg/kg lanthanum nitrate group. Indeed, it seems that REEs always have a positive and negative biological effect. For example, they can promote neurite formation and increase cell adhesion[2], but also cause nephrogenic systemic fibrosis[16]. The mechanism underlying both conditions was the same in those studies, but the effects were opposite, possibly due to differences in study subjects, exposure time, and methods of exposure. Thus, additional basic research will be required to determine the thresholds of adverse effects of REEs.

The study of the developmental neurotoxicity of REEs is not new, and some research has already reported that REEs could be harmful for memory and interfere with the release of neurotransmitters; however, the exposure time in those studies was much longer than is recommended by OECD guideline 426. Using the Morris water maze test, Feng and colleagues[3] found that rats exposed to lanthanum through oral administration of 40 mg/kg from GD 0 through 5 months of age show signs of significantly impaired memory and learning ability. He and colleagues came to a similar conclusion using a longer duration of lanthanum exposure: from GD 0 to 6 months of age[4]. Given the potential for perturbations during nervous system development to result in long-term irreversible consequences in the structure and function of the nervous system, and given that the most vulnerable period in the development of the central nervous system is during the prenatal and early postnatal stages of life[17], we exposed pups to $Y(NO_3)_3$ from GD 6 to PND 21 to see if any untoward effects occurred during the early postnatal stages of development. We found no significant differences in learning, memory, or probe trials, however. We suggested two possible explanations for this. First, REEs toxicity following oral administration is not high[1]; thus, the exposure time recommended by OECD guideline 426 may not have been long enough for the amount of REEs accumulating in the pup brains to reach the threshold required to cause any damage. Second, perhaps the cognitive function tests, conducted in adolescent and young adult rats, were carried out too early to pick up signs of the memory loss or decrease in learning ability that is associated with aging.

In conclusion, our results demonstrate that exposure to yttrium nitrate during pregnancy and lactation period can cause a significant increase in body weight in both male and female rat pups over a limited period of time (PND 21-PND 35); body weight continues to increase thereafter but at a nonsignificant rate. Except for the groups given 5 mg/kg daily, the duration of female forelimb grip strength and ambulation were decreased on PND 13. However, yttrium nitrate exposure did not affect measures of behavioral ontogeny or pre-weaning and post-weaning behavior. Thus, exposure to elevated doses of yttrium nitrate (45 mg/kg) does not appear to have adverse effects on neurobehavioral outcomes during the early stages of development in rats.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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