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Behavioral Changes in Juvenile Rats After Prenatal Exposure to Ethosuximide

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NAKANISHI, H. AND T. FUJII. *Behavioral changes in juvenile rats after prenatal exposure to ethosuximide.* PHARMACOL BIOCHEM BEHAV **36**(1) 163–168, 1990.—Juvenile rats 4–6 weeks old exposed prenatally at days 5–20 of gestation to ethosuximide at 10 mg/kg/dams' body weights per day were examined for behavioral abnormalities. Pinning behavior in the pups aged 4–5 weeks was significantly more frequent than that in age-matched controls. However, basal activity of open-field behavior and activity inhibited by diazepam administration in the pups aged 5–6 weeks showed no difference from the controls. The intensity of stereotyped behavior induced by apomorphine (1 mg/kg, SC) was significantly greater in the pups aged 5–6 weeks than in the controls. These results indicate that prenatal exposure to ethosuximide may cause changes in the dopaminergic neurons in the central nervous system.

Ethosuximide Offspring rats Play-fighting Stereotypy Open-field

MEDICATIONS given to pregnant women are usually continued throughout pregnancy, and convulsive seizures are usually treated by prescription of more than one drug (7). The ingestion of many anticonvulsants long-term during pregnancy can cause malformations in the offspring (7, 8, 25). Ethosuximide has few teratogenic effects on human (7,8) and mice fetuses (25) when given long-term during pregnancy. However, large doses of this drug administered to pregnant rats increase the incidence of malformation (9). The effects of prenatal exposure to ethosuximide on postnatal functional development in the central nervous system (CNS) of offspring of experimental animals have not been reported.

There can be abnormalities in functional development without morphological damage to offspring exposed during the prenatal period through maternal medication to drugs acting on the CNS (1,5). Exposure to phenytoin, chlorpromazine, haloperidol, or imipramine during the prenatal period leads to developmental dysfunctions of the CNS in the rat pups (11, 12, 14). Behavioral analysis is valuable for detection of subtle changes in the CNS function of rat pups when drugs are given to their pregnant mothers (1, 5, 6). Exposure to diazepam during mid-gestation results in transient hyperactivity in the open field (10). Early postnatal exposure to haloperidol via the rat mother's milk significantly increases play-fighting in juvenile rats, but not the activity in the open-field behavior (20). Play-fighting is the most frequent form of social interaction among juvenile rats (22,27).

Ethosuximide significantly decreases apomorphine-induced climbing behavior in a dose-dependent way (15), and antagonizes the action of methamphetamine which can cause stereotyped behavior (16). Ethosuximide moves through the placenta after being given to the mother (21). Hence, treatment with this drug during pregnancy may affect the functional development of dopaminergic neurons in the CNS of the offspring.

The effects of exposure in utero to ethosuximide on play-fighting, open-field behavior, and stereotyped behavior in juvenile rats were investigated in this study.

METHOD

Subjects and Treatment

Primiparous Wistar-Imamichi female rats 8–9 weeks of age were obtained from the Institute of Animal Reproduction, Saitama, and housed 2 per cage upon arrival. The animals were kept in a temperature-regulated room (24.0 ± 1.5°C) under a 14/10-hr light-dark cycle (lights off at 22:00 hr) with food and water available ad lib. Vaginal smears were taken daily. On proestrus, females were paired overnight with a sexually experienced male rat of the same strain. The presence of sperm in the vaginal smear on estrus was defined as day 0 of gestation. All pregnant rats were randomly assigned to one of three groups: 1, a group given no treatment; 2, a group given distilled water (the solvent used in the third group); and 3, a group given ethosuximide. Starting on day 5 of gestation and continuing to day 16, the pregnant rats in groups 2 and 3 were given SC daily 1 ml/kg distilled water or 10 mg/kg ethosuximide (Sankyo), respectively. All pregnant rats were weighed on days 5, 8, 11, 14, 17, and 20 of gestation, and housed in individual cages starting 1 week before parturition. On postnatal day 2, the pups in each litter were counted, their sex was identified, and the pups were redistributed to foster dams. Cross-fostering involved removal of all pups from their biological mothers and their placement with another lactating female from group 1 that had given birth on the same day. Pups from dams given distilled water were kept together, as were those from dams given ethosuximide. However, siblings were not kept together, but were assigned randomly to foster dams. Each foster dam was given 6 male and 6 female pups. In this study, data were collected only from pups

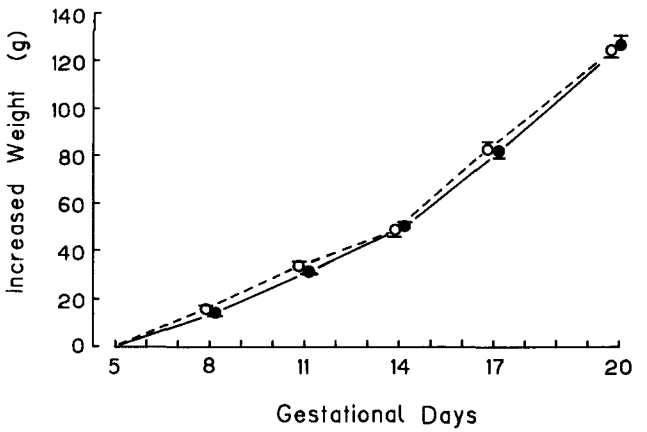

FIG. 1. Mean weight gain in the pregnant rats given ethosuximide (●; 10 mg/kg, SC) or distilled water (○; 0.01 ml/kg, SC) once daily for 16 consecutive days starting on the 5th day of gestation. The weight gain is shown as the difference from that on day 5 of gestation. Mean weights on day 5 were 299.4 ± 7.4 g in the group given ethosuximide and 306.7 ± 6.9 g in the controls. Each value is the mean ± S.E.M. of 12 measurements. The vertical bars show the S.E.M.

born to mother rats who received either distilled water or ethosuximide during pregnancy.

The control pups and those from dams given ethosuximide were weighed on postnatal days 6, 11, 16, and 21. At 22 days of age, the pups were taken from their foster dams and housed in groups of a single sex consisting of five to six animals from the same cage. The animals were kept in these groups throughout the study.

Apparatus and Procedures

Play-fighting. At 30–35 days of age, animals were weighed, and five rats without significant differences in body weight among 6 rats of the same sex caged together starting on postnatal day 2 were used to examine gregarious play-fighting. These rats were marked with a dye for easy identification. The test apparatus was a box of clear plastic measuring 27 × 45 × 40 cm high with a floor divided into 2 compartments with a fence (1 × 40 × 8 cm high). The floor was covered with wood shavings like those used in the home cage. Illumination by a 25-W white light bulb was at 0.3 lux on the floor of the test chamber.

Five animals were removed from their cage and placed in the test chamber for observation. The number of pins, that is, where one animal is held with its back on the ground by another animal, was counted as the rats played together for 10 min. The rats were given preliminary tests of play-fighting for three consecutive days before the final test so that they would become accustomed to the test chamber. Pinning could readily be distinguished from other play-fighting behavior. This measure is correlated with more subjective measures of play-fighting (23), so we used it here as a general index of play-fighting. This test and the following behavioral tests were carried out in a small room with white background noise of 53–54 dB at 10:00–14:00.

Open-Field Behavior

When the rats were 5–6 weeks old, their activity in response to environmental stimulation was assessed in a circular open-field arena. The apparatus was made of dark-grey polyvinyl chloride with a bottom 80 cm in diameter and an enclosing wall 60 cm high. The apparatus was divided into one inner most circle, six middle parts, and 12 outer parts with concentric circles and radial segments marked on the floor. The apparatus was placed on the floor in the center of a small room illuminated by two 40-W fluorescent tubes on the ceiling. An animal was placed in the inner most circle of the open-field apparatus and two kinds of activities were recorded during 3-min periods: ambulation, which was expressed as the number of times it crossed the line, and the frequency of rearing. After observation of the basal behavior in this apparatus, the animal was injected with diazepam (1.5 mg/kg, IP) and tested starting at 10 and 30 min following the injection. The animals were returned to their home cage after the test ended. The bottom and walls of the apparatus were wiped with a damp towel before the next animal was tested.

TABLE 1

EFFECTS OF DAILY ADMINISTRATION OF ETHOSUXIMIDE OR DISTILLED WATER FOR 16 DAYS STARTING ON DAY 5 OF GESTATION IN RATS ON THE DURATION OF GESTATION, THE NUMBER OF PUPS PER LITTER AND SEX RATIO IN LITTERS

	Control Group	Treated Group
Duration of gestation	22 ± 0.2	22 ± 0.3
Number of male pups	6.83 ± 0.56	6.33 ± 0.38
Number of female pups	6.08 ± 0.53	6.50 ± 0.44
Male/female ratio	1.24 ± 0.17	1.05 ± 0.11
Total number of pups per litter	13.00 ± 0.72	12.83 ± 0.42

Mean ± S.E.M. were calculated from data for 12 litters.

Stereotyped Behavior

When the rats were 5–6 weeks old, they were placed in a clear plastic box measuring 42 × 28 × 35 cm high with paper covering the floor. Their behavior was observed for 3 min before an injection of apomorphine-HCl (1 mg/kg; SC) was given and again 10, 30, and 60 min after the injection. The paper covering the floor was changed after the end of every observation of stereotypy, and was checked for tooth marks. On the day before the test, the rats underwent a preliminary test 3 times on the same schedule with no injection, to allow adaptation to the new environment.

The intensity of stereotypy was scored as (4): 0, normal behavior; 1, discontinuous sniffing and constant exploratory activity; 2, continuous sniffing and periodic exploratory activity (with little locomotion); 3, continuous sniffing in one location (along a fixed path) and discontinuous licking or biting; 4, continuous licking or biting in one location.

Statistical Treatment

The significance of differences between the control pups and pups of mothers given ethosuximide was evaluated by use of analysis of variance, and followed by Student's *t*-test or Welch's *t*-test.

RESULTS

Maternal Body Weight and Number of Pups

On day 5 of gestation, the mean body weights of the pregnant

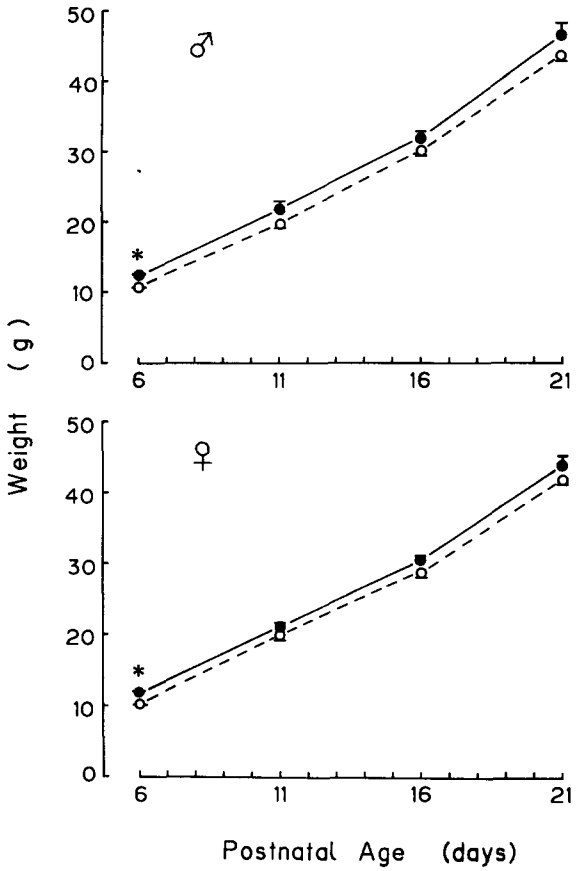

FIG. 2. Mean weight of male and female pups born to dams given ethosuximide (●) or distilled water (○) during pregnancy, and nursed by foster dams after birth. Each value is the mean ± S.E.M. of 66 determinations. The vertical bars show the S.E.E. *$p<0.05$ relative to age-matched controls.

rats in the two groups were not significantly different (299 ± 7 g in the group given ethosuximide and 306 ± 7 g in the group given distilled water). The gains in weight from days 5 to 20 of gestation in the two groups also were not significantly different (Fig. 1). The length of gestation, number of offspring delivered, and the sex ratio within litters were not significantly affected by the administration of ethosuximide (Table 1).

Body Weights of Pups and Foster Dams During Lactation

The mean body weight gains by offspring during nursing by foster dams from postnatal day 6 until weaning on postnatal day 21 were compared in the two groups. On day 6, the mean body weight of pups whose mothers had been treated with ethosuximide were significantly greater than their age-matched controls ($p<0.05$). However, the significance of the difference disappeared later (Fig. 2). The mean body weights of foster dams nursing pups exposed prenatally to ethosuximide were 348 ± 6, 350 ± 6, 359 ± 7, and 345 ± 7 g on days 6, 11, 16, and 21 after delivery. The means of foster dams nursing control pups were 341 ± 11, 350 ± 11, 358 ± 10, and 351 ± 8 g on these days. The differences were not significant.

Pinning Behavior

The effects of prenatal exposure to ethosuximide on pinning

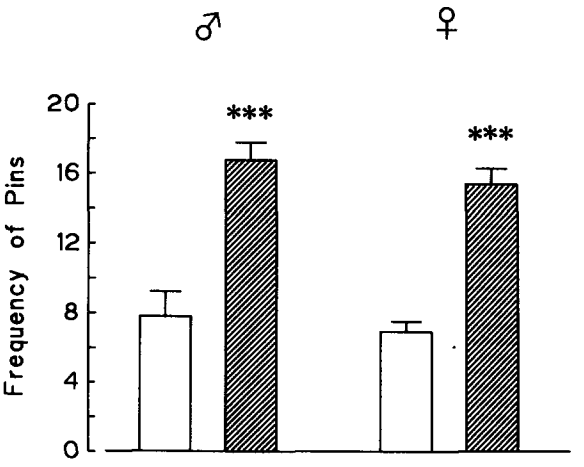

FIG. 3. Number of pins during 10-min observations of 5 rats together. The pups exposed prenatally to ethosuximide are represented by hatched columns and those exposed to distilled water by solid columns. Each value is the mean ± S.E.M. of 10 determinations. The vertical bars show the S.E.M. ***$p<0.001$ relative to age-matched controls.

behavior in a total of 220 pups at 4–5 weeks of age under the gregarious environment of five rats grown in the same cage are shown in Fig. 3. The mean body weights of male and female pups whose dams were treated with the drug were 106 ± 3 and 102 ± 3 g. The means for the male and female control pups were 98 ± 3 and 93 ± 2 g. In each group, the difference between the smallest and largest of the five rat pups tested did not exceed 10 g. The frequency of pins observed in 10 min in the male and female rats whose dams were given ethosuximide was significantly more than in the male and female control rats ($p<0.001$ for both sexes). Pinning behavior was elicited by attacks by all five rats, not by attacks of only one or two rats.

Basal Open-Field Behavior and Behavior With Diazepam Administration

The effects of diazepam on open-field behavior in male and female rat pups at 5–6 weeks of age are shown in Fig. 4. The rats used in this experiment did not receive the test of play-fighting behavior. Exposure in utero to ethosuximide did not significantly change the basal frequency of ambulation or rearing in either sex. Nor did it affect the incidence of these behaviors when inhibited by diazepam.

Stereotyped Behavior

The effects of exposure to ethosuximide in utero on stereotyped behavior induced by apomorphine in rat pups are shown in Fig. 5. The stereotypic responses in the male pups of dams given ethosuximide were significantly more intense 10 and 30 min after apomorphine treatment compared to the controls ($p<0.01$ and $p<0.05$ at the two times). The stereotypic responses in female pups of dams given ethosuximide had also increased significantly at 30 min after apomorphine injection compared to the controls ($p<0.05$).

DISCUSSION

There is little evidence of an association between fetal malformations and maternal intake of ethosuximide. Maternal rats given

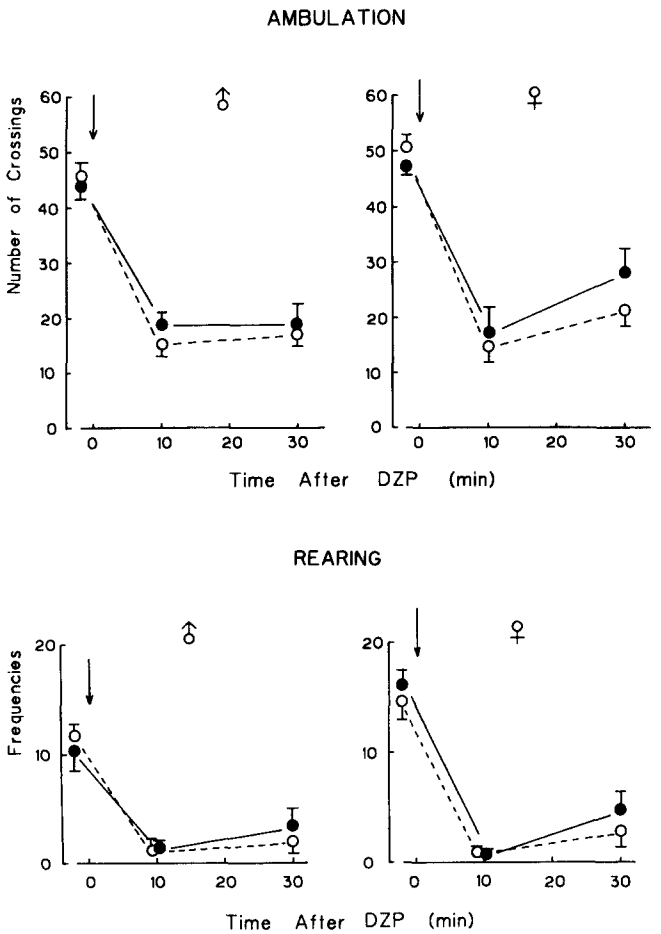

FIG. 4. Open-field behavior (ambulation and rearing) in male and female pups exposed prenatally to ethosuximide (●) or distilled water (○). The arrows show the injection of diazepam (DZP). Each value is the mean ± S.E.M. of 9 determinations. The vertical bars show the S.E.M.

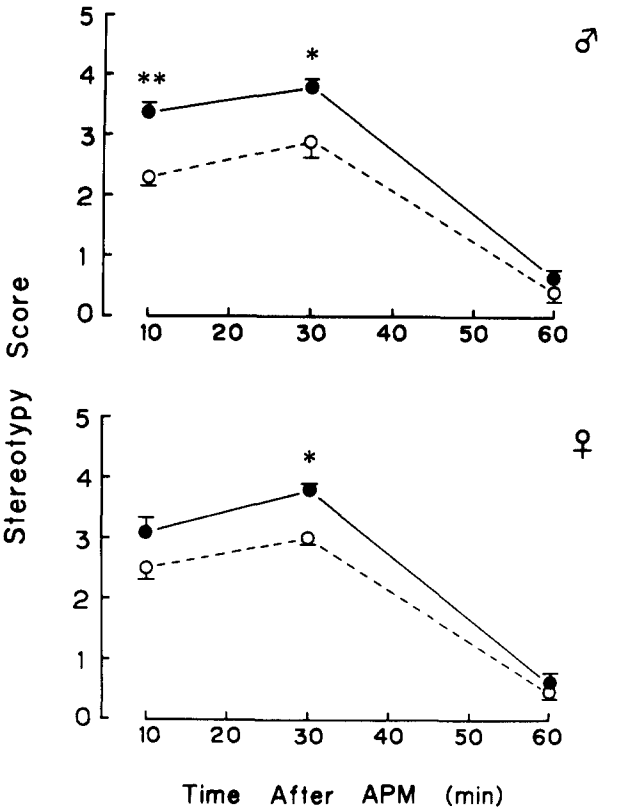

FIG. 5. Intensity of stereotyped behavior induced by apomorphine (APM) in male and female pups exposed prenatally to ethosuximide (●) or distilled water (○). Each value is the mean ± S.E.M. of 10 determinations. The vertical bars show the S.E.M. *$p<0.05$ and **$p<0.01$ relative to age-matched controls.

a higher dose of this drug (250 mg/kg) than was used here during the period of embryonal organogenesis had fetuses that weighed less than the controls on day 19 of pregnancy; also, the number of live fetuses per litter and the crown-rump length of the fetuses were less than in the controls (9). The fetuses of pregnant mice given ethosuximide (180 or 360 mg/kg) daily by gastric intubation were not significantly changed in these ways (25). These different results suggest that these species have different thresholds for the development of teratogenic effects as a result of large doses of ethosuximide. Here, prenatal administration of a low dose (10 mg/kg, SC) of ethosuximide during days 5–20 of pregnancy did not have significant effects on the duration of gestation, the weight gain of the pregnant rats up until delivery, the number of pups per litter or the ratio of male to female pups. These findings suggest that the food and water consumption of pregnant rats and the growth of fetuses were not influenced by repeated injections of ethosuximide into the dams. However, food and water intake was not measured.

Behavior may indicate small changes in the functioning of the CNS caused by exposure to doses of drugs and chemical substances lower than those that produce neurotoxicity (1, 5, 10). When the compound is given during pregnancy, behavioral changes that appear in the offspring are mediated through both prenatal mother-fetal and postnatal mother-offspring interactions.

The behavior of adult rats can be influenced by different handling of their mothers by the experimenter during the infancy of the offspring (5). The development of the mouse brain is influenced by postnatal nutrition (3) and the maternal environment (13). Ethosuximide is found in breast milk of mothers of several species following treatment of the mother with the drug, and the ratio of milk/serum concentrations is 0.9, so nursing neonate could ingest considerable amounts of the drug via breast milk (21). These results suggest that the changes in the mother's milk and the mother's general and feeding behavior induced by drug treatment might influence the behavior of the offspring. In this experiment, therefore, cross-fostering was used so that a distinction could be made between the pre- and postnatal effects of ethosuximide. Also, the number of offspring in litters given to foster dams were limited to 12 so that the nutritional intake by the pups would be more uniform than with litters of different sizes. In this study, body weight gains in pups during lactation were significantly different only when measured for the first time on day 6 after birth. Since the half-life of clearance of transplacentally acquired ethosuximide in one human neonate was 41.3 hr (17), this result suggests that the increase in body weight in pups whose dams are treated may be due to changes in suckling related to withdrawal from ethosuximide.

Prenatal administration of drugs affects the open-field behavior of the offspring of several species (5). In this study, prenatal exposure to ethosuximide did not influence basal ambulation or rearing in offsprings at 5–6 weeks of age. Diazepam accelerates the functioning of the γ-aminobutyric acid (GABA) system in the

brain (19), while ethosuximide antagonizes the inhibition of glutamate decarboxylase activity and the decrease in GABA levels caused by isoniazid (18,19). These findings suggested that ethosuximide may have an effect on the GABAergic system similar to that of diazepam. In the present result, the inhibition of ambulation and rearing by diazepam was not significantly different between rats whose dams were given ethosuximide and those which were not. In addition to this finding, ethosuximide given to rats for 10 days at 150 mg/kg per day had little effect on levels of GABA in the whole brain or in regions of the brain (24). Therefore, prenatal exposure to ethosuximide may have no effect on the GABAergic system accelerated by diazepam.

Social play (play-fighting) in juvenile rats is a sensitive measure of normal behavioral interaction and has been used as a model in investigations of a variety of psychobiological variables (15). In the analysis of the juvenile social play with isolated pairs of rats, the heavier juveniles tend to become dominant because they can more easily tip over and pin down a companion (22,27). In this study, the effect of prenatal exposure to ethosuximide on normal behavioral interaction was estimated by observation of pinning behavior, a simpler indicator of play-fighting behavior, by five rats housed together starting when they were 2 days old. Moreover, the difference in weight between the largest and smallest rats did not exceed 10 g in this test of gregarious behavior. Pinning behavior in both sexes of 4–5-week-old pups of dams given ethosuximide was significantly more frequent than that in the controls. This finding was in agreement with our previous findings that postnatal treatment with haloperidol via rat mother's milk during lactation significantly increased play-fighting behavior of rats at 4 weeks of age (21). Thus, long-term treatment with haloperidol or ethosuximide during the development of the CNS of rats may enhance play-fighting behavior.

The addition of ethosuximide antagonized the stereotyped behavior induced by methamphetamine (16). Apomorphine antagonized catalepsy induced by a high dose (400 mg/kg) of ethosuximide in a dose-dependent way (15). In mice, ethosuximide significantly decreased apomorphine-induced climbing behavior in a dose-dependent way (15). These findings show that ethosuximide might antagonize the increase in dopaminergic responses induced by apomorphine and methamphetamine. Stereotypic responses induced by apomorphine in pups of both sexes whose dams were given ethisuximde long-term were more intense than in the controls. Juvenile rats deprived of social stimulation by prolonged isolation show increased play-fighting (2), accompanied by a decrease in the steady-state levels of norepinephrine and dopamine in the brain (26).

These findings suggest that prolonged exposure to ethosuximide in utero may increase the sensitivity of the dopaminergic system in the CNS of the developing rats, and that play-fighting (pinning behavior) is a sensitive index of functional abnormalities in the CNS in juvenile rats.

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