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Prenatal Antiepileptic Drug Exposure Alters Seizure Susceptibility in Rats

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SOBRIAN, S. K. AND A. K. N. NANDEDKAR. *Prenatal antiepileptic drug exposure alters seizure susceptibility in rats.* PHARMACOL BIOCHEM BEHAV **24**(5) 1383–1391, 1986.—An animal model is used to address the issue of prenatal exposure to certain antiepileptic drugs and seizure susceptibility in the offspring. Administration of doses established as median therapeutic doses in humans of phenobarbital, valproate and clonazepam to pregnant rats during the last third of gestation produced sexually dimorphic alterations in pentylenetetrazol (PTZ)-induced seizures as well as in non-convulsive (spontaneous alternation and cliff avoidance) behaviors in the offspring. Altered seizure susceptibility occurred in the absence of overtly recognizable morphological abnormalities and did not appear to reflect differences in the status of circulating drug-binding plasma proteins. Possible neural and/or metabolic mechanisms responsible for these behavioral changes are discussed.

Prenatal drug exposure Phenobarbital Clonazepam Valproic acid Pentylenetetrazol
Convulsive behavior Non-convulsive behavior

CLINICAL and experimental evidence indicates that some antiepileptic drugs (AEDs) are teratogenic. Exposure during pregnancy to phenytoin [2, 17, 18, 20, 27] or trimethadione [12,65] has resulted in growth retardation, dysmorphology of the facies and CNS dysfunction in the fetus. A similar fetal anticonvulsant syndrome has also been reported for phenobarbital, but without dysfunction of the CNS [49,54]. Valproate which is clearly teratogenic in mice, rats and rabbits [3, 4, 63], has recently been found to produce facial, digital and skeletal abnormalities as well as developmental delays in the human fetus [1, 14, 46].

A growing body of evidence indicates that *in utero* exposure to a variety of other centrally acting drugs can produce adverse behavioral effects in the offspring [23, 41, 51, 52, 53, 61, 67], many of which persist into adulthood [21, 32, 54]. Despite this fact and the link between developmental abnormalities and AEDs during pregnancy, the question of long-term behavioral sequelae or subsequent seizure susceptibility following prenatal exposure to AEDs has, until recently, received little attention.

The results of animal experiments indicate that prenatal exposure to anticonvulsant medication can alter the behavior of both developing and mature offspring. Delays in the development of several motor behaviors and the acoustic startle response have been reported following prenatal exposure to phenytoin [11,60], phenobarbital [60,66] and trimethadione [60]. Prenatal diazepam administration eliminates the potentiation of rat locomotion and the acoustic startle reflex that normally appear in the third postnatal week [25]. As adults, the offspring of mice treated with phenobarbital daily during the last third of gestation do not perform in an operant situation to obtain a food reward and are unresponsive to appetitive stimuli [23]. Other studies [13,34] indicate that phenobarbital exposed offspring are also less responsive to aversive stimuli. Similar results have been reported for phenytoin; administration of this drug during the last third of gestation disrupts the acquisition of a conditioned avoidance response [13]. Changes in adult activity have also been reported following prenatal phenobarbital [35,36], phenytoin [37] and valproate [5].

Data concerning seizure activity in offspring prenatally exposed to AEDs are limited and contradictory. An early report [38] indicated that offspring exposed to phenobarbital on gestational days (GD) 5–8 displayed a significantly higher threshold for and shorter duration of electroshock-induced seizures. In contrast, administration of phenobarbital late in pregnancy (i.e., GD 17–20) failed to alter seizure thresholds in the offspring [38]. Treatment with phenytoin on GD 14–20 produced a decrease in seizure thresholds in the offspring that was evident during the first 3 postnatal weeks; after this time, thresholds returned to control levels [57,58].

We conducted a study to determine if prenatal exposure to phenobarbital, valproate or clonazepam, in doses established as median therapeutic doses in humans, would protect or sensitize the offspring to the convulsant action of pentylenetetrazol (PTZ). Our working hypothesis was that exposure of the developing brain to AEDs in non-teratogenic doses could induce long-term changes in the central nervous system (CNS) which may contribute to alterations in seizure susceptibility. During neuronal differentiation, brain tissue may be particularly sensitive to drugs that act on the CNS to produce alterations that are later expressed as changes in behavior. The question of whether the consequences of prenatal AED exposure elaborates identical responses in both sexes was also investigated, since gestational manipulation can differentially affect the behavior of male and female off-

spring [5,36] and seizures can be triggered or exacerbated by puberty [22, 41, 50, 56].

METHOD

Animals and Drugs

Thirty-six timed-pregnant Sprague-Dawley rats (Charles River Laboratories, Somerville, MA) were delivered to our laboratory on gestation day 7 (GD 7); the morning that a vaginal plug was found was designated GD 1. Females were housed individually in polyethylene maternity cages under environmentally controlled conditions (0800 hr light, 2000 hr dark; ad lib access to Purina Rat Chow and water; ambient temperature 23–25°C). Nine females were randomly assigned to each of 3 drug groups and 9 females served as vehicle controls.

On GD 15–20, females were treated daily with either phenobarbital (Phenobarbital, 15 mg tablet, Rexall), clonazepam (Clonapin, 0.5 mg tablet, Hoffman-La Roche), or valproate (Depakene, 250 mg capsule, Abbott). These drugs were chosen because they are (1) chemically and pharmacologically unrelated, (2) effective against PTZ-induced seizures, and (3) effective individually as therapeutic agents in human epilepsy.

The developmental window was selected because we have found that administration of drugs on the expected day of delivery (GD 21) is disruptive to maternal behavior. GD 15–21 is the period of the fetus in the laboratory rat, a time during development when the embryo becomes increasingly refractory to gross structural malformations. Agents administered after GD 15, as organogenesis becomes complete, are unlikely to produce gross defects. They can, however, produce subtle damage in the central nervous system by interfering with histogenesis in the cortex, forebrain, cerebellum and hippocampus where cells are actively proliferating at this time. Exposure to xenobiotic agents can either kill the dividing cells outright or slow the rate of proliferation, thereby disrupting subsequent differentiation and migration. Those areas of the brain sustaining cell loss fail to function normally with resulting behavioral alterations.

Daily drug doses used were those established as median therapeutic doses in humans: phenobarbital, 2.0 mg/kg; clonazepam, 0.15 mg/kg; and valproate, 20 mg/kg. Human therapeutic doses have been used previously to evaluate prenatal AED effects [40,42] and were chosen in an effort to minimize or eliminate the production of gross teratogenicity and side effects such as sedation which might interfere with food and water intake or parturition. Use of these doses, however, does not imply that either the median therapeutic anticonvulsant doses in humans and rats are equivalent, or that the same dose levels will produce equivalent plasma levels of the drug in the two species. The 6–10 fold difference in metabolic rates between rodents and man necessitates a 17 fold increase in the milligram/kilogram dose of phenytoin needed to achieve plasma levels in the rat comparable to those in the human therapeutic range [28]; a similar increase in the dose of valproate (i.e., 18 fold) is needed in mouse [40]. However, administration of these elvated drug doses on a once daily injection schedule substantially increases the risk of teratogenicity [40].

Drugs were suspended in propylene glycol and injected subcutaneously at the back of the neck once daily between 1000 and 1200 hours; injection volumes ranged from 0.25–1.00 ml. Controls were injected with propylene glycol, 0.1 ml/100 g body weight. Following AED injections, females were monitored for maternal toxicity. Animals were returned to their cages and the appearance of sedation and ingestive behaviors were recorded (present or absent) using a time sampling method (i.e., once every 10 minutes for one hour and successively once per hour for four hours). Females were allowed to deliver naturally. Litters, raised by their biological mothers, were culled to 10 pups at birth to insure adequate nutritional status. Body weights of the offspring were recorded at birth and every 7 days until weaning at postnatal day 28 (PND 28). Offspring were housed in like-sex groups of 3–5 animals in wire mesh cages.

RATING	DESCRIPTION
0	Normal behavior: Locomotion, rearing, grooming.
1	Excessive grooming, sniffing, licking, chewing, salivating.
2	Running, hopping movements, digging, kicking with hind limbs.
3	*Minimal Threshold Seizures:* Facial clonus, rhythmic movements of vibrissae, jaws and ears lasting for five seconds.
4	*Submaximal Seizure:* Mild clonic movements of head and forelimbs.
5	Tonic extention of forelimbs and/or hind limbs.
6	*Maximal Seizure:* Generalized asynchronized, clonic movements, superseded by a tonic convulsion.

Behavioral Testing

Convulsive behavior. At 34–38 days of age offspring from each of the four prenatal treatment groups were tested for their response to one of three doses of IP pentylenetetrazol (PTZ: Sigma Chemical Company); 20 mg/kg, subthreshold convulsive dose; 35 mg/kg, minimal threshold convulsions; 50 mg/kg, maximal convulsions.

PTZ was dissolved in 0.9% NaCl and the injection volume was 0.5 ml/100 g body weight. The control group consisted of offspring from each of the 4 prenatal groups who were injected IP with 0.9% NaCl (0.0 mg/kg). Animals were monitored continuously for 30 minutes and convulsive behavior was rated using the 7 point scale listed below. Two observers, one blinded with respect to prenatal treatment and the other blinded with respect to dose of the seizure inducing agent, independently recorded convulsive behavior. The highest rating recorded for an animal during this test period was designated its convulsive behavior score (CBS). *Each pup was tested only once.*

Nonconvulsive behavior. Spontaneous motor activity (SMA) was monitored in the offspring from the four prenatal groups injected with 0.9% NaCl. Following injection, animals were placed individually in a polyethylene container (44×25×30 cm) which contained 800 ml of pine shavings. The container was placed on top of an automated activity meter (Automex II, Columbus Instrument International Corporation, Columbus, OH), which was adjusted to record only lateral excursions. SMA was monitored for 30 minutes. Activity was not measured in animals treated with PTZ because the appearance of convulsive behavior would preclude locomotion. At 43–45 days of age, naive sets of offspring

were tested for the appearance of two nonconvulsive behaviors: spontaneous alternation and cliff avoidance.

Spontaneous alternation (SA). This is an unlearned task which reflects the development of inhibitory circuitry in the CNS, primarily in the hippocampus [8]. It requires that a rat, on two successive unrewarded trials in a T-maze, choose alternate arms of the maze.

Testing was conducted in a black Plexiglas T-maze. A sectioned hinged clear Plexiglas lid permitted separate access to the start box, the main alley and the 2 goal arms. The main alley measured 11.5×35.0×11.5 cm. A 15 cm section of this alley served as a start box and was separated by a black guillotine door. The goal arms measured 11.5×25.0×11.5 cm; black plastic guillotine doors separated the goal alley from the main alley. The maze was housed in a small windowless room; a shaded 25 watt red light bulb suspended 25 cm above the choice point of the maze, provided the only illumination. Each alternation test consisted of two trials. A trial consisted of placing the animal in the start box for 10 seconds. The guillotine door was then raised and the rat was given 3 minutes in which to enter one of the goal arms with all 4 paws. The rat was then left in the chosen arm for 50 seconds after which it was removed to a holding cage for 60 seconds. During this intertrial interval, the maze was wiped with a mild solution of Micro brand laboratory cleaner (International Products Corporation) to eliminate odor trails. Alternation behavior was scored once for each animal. Animals were not given pretest training and a reward was not used during testing.

SA exhibits a developmental pattern; preweanling rats will choose the same goal arm on both occasions (perseverative behavior). Change levels are seen at approximately 3–4 weeks of age. Reliable alternation behavior has been reported in animals between 35–40 days of age [8, 9, 10].

Cliff avoidance (CA). Depth perception, a sensory function, was assessed with a visual cliff apparatus (Lafayette Instrument Company, Lafayette, IN). The apparatus is a box (75 cm square × 60 cm high). A sheet of glass, suspended 20 cm from the top, covers the adjustable floor, which is divided in half; one side of the floor extends 40 cm below the glass and the other 5 cm below the glass. The sides and floor of the apparatus are covered with a gray and red checkerboard pattern. In the middle of the apparatus there is a runway 10 cm wide and extending the length of the box; the top of the runway is 5 cm from the glass covering. At the beginning of the trial, a rat is placed in a rectangular box (10×25×10 cm) on the runway. After 15 seconds the box is removed and the latency to choose and side chosen are recorded. An animal demonstrates depth perception by choosing the shallow side of the test apparatus.

Biochemical Determination

Serum protein determinations were made in separate groups of offspring. The possibility exists that alteration in drug binding might be responsible for change in seizure susceptibility in the offspring. Proteins were analyzed to provide an *indirect index* of binding capacity. Trunk blood was used on postnatal days 0–1 and 7; 1.0 ml of blood obtained from the tail vein of unanesthesized animals was used for day 30 assays. Total proteins were assayed by the colorimetric method of Lowry, *et al.* [29]. Serum protein fractions were quantitated by separation and staining on Universal agarose film at pH 8.6. A Corning power supply with constant voltage (15 V/cm) in a cassette system was used to separate serum proteins. One microliter of serum was applied at the cathodic end and electrophoresis run was for 35 min. One percent Amido Black 10 B in 5 percent acetic acid was used for detection and staining of the separated protein fractions. The background stain was cleared by sufficient 5 percent acetic acid and the film was uniformly dried in an incubator-oven (Corning ACI) at 60°C. Albumin/globulin ratio was calculated by electrophoretic scans. Similarly, individual protein fraction, i.e., alpha$_1$, alpha$_2$, and beta-globulins, were calculated by integration of peak area under each stained band with an Ortec densitometor (Model 4301). Control serum was run with each film to calibrate the staining intensities. Two parameters were calculated: (1) the albumin/globulin (A/G) ratio, to determine the status of circulating plasma proteins; and (2) albumin, as a percent of total proteins, which reflects the level of primary binding protein.

Statistical Methods

Completely randomized one-way analyses of variance (ANOVA) were used to analyze pregnancy weight gain, period of gestation, litter size, and birth length. Offsprings' body weight data were analyzed by a split-plot factorial ANOVA. The unit of analysis was the individual offspring [55]. A completely randomized factorial ANOVA was used to analyze convulsive behavioral scores and spontaneous motor activity. The Newman-Keuls statistic was used for significant main effect comparisons; simple main effects were used to dissect significant interactions [26]. For convulsive behavior and locomotor activity, the unit of analysis was individual offspring. One male and one female offspring from 5 litters were used. At data points with 6 animals, no more than 2 males or 2 females per litter were used.

Spontaneous alternation data were first analyzed for position preference in order to determine the *a priori* probability of alternation for each treatment group [7]. The probability of alternation is equal to 1 (percent of responses to preferred side^2 + percent of responses to non-preferred side^2). This value was then used in the Chi Square statistic to determine whether alternation behavior was statistically different from chance in each group. Latencies for cliff avoidance behavior were analyzed by a completely randomized 2-way ANOVA. The percentage of offspring in each group exhibiting CA were analyzed by Chi Square. Percentage data for both SA and CA were analyzed using *both* the individual and litter as the unit of analysis; these latter data are presented for comparison.

RESULTS

Maternal Toxicity and Birth Statistics

Maternal toxicity was not observed in either control or drug-treated females. Time-sampling observations did not reveal any drug-related sedation, and food and water intake appeared comparable in all groups. However, an irritation developed at the injection site in 1 phenobarbital-treated female after 3 days of treatment and in 1 valproate-treated female after 4 days. Weight gain during the last third of gestation and length of gestation (Table 1) were not significantly different, $F(3/28)=2.07$, $p>0.05$; $F(3/28)=0.04$, $p>0.05$, respectively.

Post partum litter data also appear in Table 1. Litter size, length at birth and the male/female offspring ratio were unaffected by exposure to AED on GD 15–20. Physical abnormalities, determined by gross inspection of limbs and snout,

TABLE 1

GESTATIONAL AND BIRTH STATISTICS (MEAN ± S.E.M.) OF FEMALES TREATED WITH ANTIEPILEPTIC DRUGS ON GESTATION DAYS 15–20

Females	N	GD15–20 Weight Gain (Grams)	Periods of Gestation (Days)	Litter Size
Control	8	55.88 ± 3.17	22.13 ± 0.13	11.62 ± 1.05
Clonazepam	8	47.60 ± 3.38	22.13 ± 0.13	9.62 ± 0.38
Phenobarbital	8	64.56 ± 3.67	22.38 ± 0.18	11.12 ± 0.77
Valproate	8	52.4 ± 7.98	22.13 ± 0.13	11.12 ± 0.52

Litters	N	Male/ Female Ratio‡	Birth Weight (g) Males	Females	Birth Length (cm)
Control	8	1:1.11	5.64 ± 0.21†	5.24 ± 0.22	4.96 ± 0.08
Clonazepam	8	1:1.17	6.37 ± 0.19*†	5.91 ± 0.24*	4.81 ± 0.11
Phenobarbital	8	1:0.93	5.80 ± 0.23†	5.05 ± 0.14	4.90 ± 0.27
Valproate	8	1:0.96	5.90 ± 0.25†	5.54 ± 0.11	4.95 ± 0.09

*Significantly different from controls, $p<0.01$.
†Males significantly different from females, $p<0.01$.
‡The male/female ratio was calculated from total number of offspring in each treatment group.

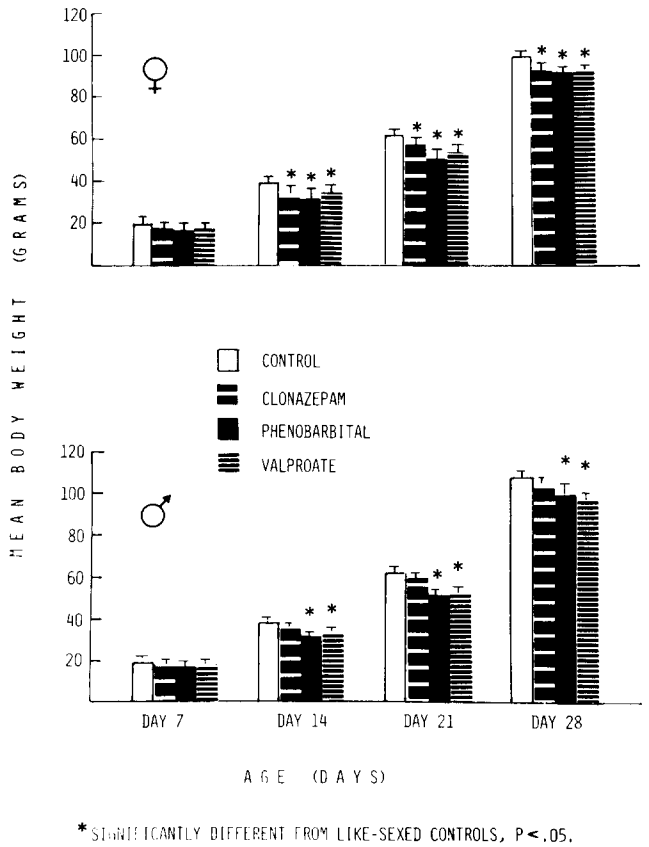

FIG. 1. Postnatal preweanling body weights of offspring of females treated on GD 15–20 with either phenobarbital, clonazepam or valproate. Values shown are mean±S.E.M.

were not observed in the offspring at birth and did not develop. One clonazepam pup was stillborn; no further deaths occurred.

Postnatal body weights. At birth, male offspring in all 4 groups were heavier than females (Table 1), $F(1/32)=5.07$, $p<0.01$; no differences were observed at PND 7 (Fig. 1). At PND 14, 21, and 28 male and female offspring of phenobarbital and valproate mothers were significantly smaller than controls, $F(3/407)=4.48$, $p<0.01$; $F(3/407)=10.01$, $p<0.01$. Although female clonazepam offspring were significantly smaller than control females, the body weights of their male counterparts did not differ from those of control males. The sexual dimorphism in body weight (males are heavier than females) which appears during the 3–4 postnatal week was evident in all offspring, except those from valproate dams, at PND 28.

Seizure susceptibility. The effects of prenatal exposure to phenobarbital, clonazepam or valproate on PTZ-induced convulsions are reported in Table 2. Neither saline (0.0 mg/kg) nor the subthreshold dose of PTZ (20 mg/kg) produced convulsions in any of the offspring. Most animals exhibited either normal behavior (CBS=0) or showed excessive grooming (CBS=1); several offspring in all groups did show isolated hind limb kicking (CBS=2) following 20 mg/kg of PTZ.

The offspring of control mothers exhibited minimal threshold seizures (CBS=3) at 35 mg/kg of PTZ. In contrast, none of the offspring of clonazepam, phenobarbital and valproate treated mothers exhibited minimal threshold seizures at this dose; CBSs were significantly lower in these 3 groups than in controls, $F(3/35)=4.10$, $p<0.05$.

The most striking difference among the groups occurred in their responses to the 50 mg/kg dose of PTZ, and reflected an interaction between prenatal AED exposure and the sex of the offspring, $F(3/35)=4.95$, $p<0.01$. A simple main effect

TABLE 2

CONVULSIVE BEHAVIOR SCORE (CBS) TO PTZ FOLLOWING PRENATAL EXPOSURE TO AEDs

Prenatal Treatment		Dose of PTZ 0.0 mg/kg	20 mg/kg	35 mg/kg	50 mg/kg	Latency‡ (sec)	Duration‡ (sec)	% Animals¶ With CBS 5
Control	M (5–6)†	1.00 ± 0.00	2.00 ± 1.00	3.00 ± 0.00	6.00 ± 0.00	160.00 ± 20.00	59.00 ± 39.60	100
	F (5–6)	1.00 ± 0.00	1.67 ± 0.33	3.00 ± 0.00	6.00 ± 0.00	222.50 ± 102.50	75.25 ± 2.25	100
Clona-	M (5)	0.67 ± 0.33	1.20 ± 0.20	1.00 ± 0.71*	3.25 ± 1.25*	147.5 ± 32.50	403.00 ± 64.00*	20
zepam	F (5–6)	0.67 ± 0.33	1.00 ± 0.00	1.25 ± 0.63*	4.00 ± 1.08*	189.67 ± 25.89	15.33 ± 6.01*	33.3
Phenobar-	M (6)	1.00 ± 0.00	1.67 ± 0.33	1.67 ± 0.67*	2.33 ± 0.33*			0.0
bital	F (5–6)	1.67 ± 0.67	1.00 ± 0.00	1.00 ± 0.58*	3.33 ± 0.88*	100.00§	12.00§	16.6
Valproate	M (5)	1.00 ± 0.00	1.00 ± 0.00	1.50 ± 0.50*	6.00 ± 0.00	110.00 ± 35.50	727.00 ± 96.10*	100
	F (4–5)	0.33 ± 0.33	1.33 ± 0.33	1.67 ± 0.67*	1.67 ± 1.20*			0.0

Offspring were injected IP with either saline (0.0 mg/kg) or one of 3 doses of PTZ. Convulsive behavior was monitored for 30 minutes. An animal's CBS reflected the highest rating it received during the testing period. Data presented are means ± standard error of the mean. CBS: 0=normal behavior; 1=excessive grooming, sniffing, licking, chewing, salivating; 2=running, hopping movements, kicking with hind limbs, digging; 3=Minimal Threshold Seizure: facial clonus, rhythmic movements of vibrissae, jaws and ears lasting 5 seconds; 4=Submaximal Seizure: mild clonic movements of head and fore limbs; 5=tonic extention of hind limbs; 6=Maximal Seizure: generalized asynchronized, clonic movements preceded by a tonic convulsion. Each offspring was tested only once.

*Significantly different from similar sex controls, $p<0.05$.

†Number of animals used at each dose is indicated in parentheses. At each dose of PTZ for each of the prenatal treatments, animals from 5 randomly chosen litters were evaluated. One male and one female from each litter were used; at data points with 6 animals, no more than 2 males or 2 females were used from a single litter.

‡Latency and duration (mean ± S.E.M.) for CBS of 5 or 6 induced by 50 mg/kg of PTZ.

§Data from one animal.

¶50 mg/kg dose of PTZ.

analysis revealed that prenatal exposure to phenobarbital protected both male, $F(3/17)=4.06$, $p<0.05$, and female, $F(3/18)=4.36$, $p<0.05$, offspring from PTZ-induced convulsions. The 50 mg/kg dose, which induced maximal seizures (CBS=6) in the controls, produced only minimal threshold seizures (CBS=3) in all males and in five of six females (one female exhibited submaximal seizures) (CBS=4).

The offspring of valproate-treated mothers exhibited a sexually dimorphic response to this convulsive dose of PTZ. Prenatal exposure to this antiepileptic drug protected female offspring from maximal seizures. In contrast, male offspring were not protected; they appeared to be sensitized to maximal convulsions, as the duration of the response was significantly longer than in controls ($t(9)=6.42$, $p<0.01$).

The response of pups prenatally exposed to clonazepam was also sexually dimorphic. Although the CBS was significantly lower in both males and females than in controls of the same sex, clonazepam offspring of both sex exhibited convulsive behavior following 50 mg/kg of PTZ that was rated 4 or greater. In males, the duration of the convulsions was significantly longer than in male controls ($t(9)=6.83$, $p<0.01$); in females the duration of the response was significantly shorter than that of female controls ($t(9)=9.34$, $p<0.01$).

Non-convulsive behavior. Several non-convulsive behaviors were assessed (Table 3) to determine whether the effects of the prenatal treatments were limited to changes in seizure susceptibility. If *in utero* AED exposure produced a generalized alteration in CNS development, disruptions in sensory as well as other motor behaviors could be anticipated.

Spontaneous motor activity. Changes in SMA again reflected an interaction between prenatal drug treatment and the sex of the offspring, $F(3/30)=3.37$, $p<0.05$. Male offspring prenatally exposed to clonazepam were significantly less active than their control counterparts; prenatal exposure to phenobarbital or valproate did not change SMA in males. In sharp contrast, female offspring of mothers administered phenobarbital and valproate were significantly more active than female control offspring.

Spontaneous alternation. Alternation rates for males and females in each of the four groups are presented in Table 3. No significant trial 1 direction preferences were found. Using trial 1 data, expected rates of alternation were calculated for each group using the formula of Dember and Fowler [7]. The difference between these and observed rates were calculated using Chi Square. The results are indicated in Table 3.

Reliable alternation behavior was not observed in any of the groups. This may reflect the fact that alternation behavior was scored only once for each rat [7]. However, Chi Square analysis of the percentage of offspring exhibiting SA indicated that there was a significant difference among the 4 groups for both males ($\chi^2(3)=11.93$, $p<0.01$) and females ($\chi^2(3)=12.58$, $p<0.01$). Similar results were obtained when litter was used as the unit of analysis: Males: $\chi^2(3)=18.18$, $p<0.01$; Females: $\chi^2(3)=11.92$, $p<0.01$. In the control group, males perseverated (most immature response) while females alternated at chance levels. Prenatal AED treatment altered this pattern; AED males alternated at chance levels while females exhibited perseverative behavior.

Cliff avoidance. Two dependent variables were measured: (1) the latency to make a response and (2) the side that

TABLE 3

ALTERATIONS IN SPONTANEOUS MOTOR ACTIVITY, SPONTANEOUS ALTERNATION, AND CLIFF AVOIDANCE FOLLOWING PRENATAL AED EXPOSURE

Prenatal Treatment	Spontaneous Motor Activity† (mean ± SEM Counts/30 min)		Spontaneous Alternation† (% of Animals Alternating)	Cliff Avoidance: % of Animals Exhibiting Avoidance	Cliff Avoidance: Latency (sec)
Control	1224.6 ± 41.8	M (14:4)§	28.5 (P) [22.8 ± 7.8]	42.9 [37.2 ± 14.3]	17.96 ± 6.69
	524.5 ± 364.5	F (9:3)	44.4 (C) [47.6 ± 2.4]	77.8 [79.1 ± 10.6]	7.66 ± 1.23
Clona-	578.6 ± 112.8*	M (5:3)	60.0 (C) [55.3 ± 5.3]	80.0 [72.0 ± 14.7]	11.40 ± 3.40
zepam	383.6 ± 162.3	F (9:3)	30.0 (P) [19.3 ± 9.9]	44.4 [44.4 ± 5.7]	9.22 ± 2.60
Phenobar-	1259.0 ± 99.4	M (11:4)	54.5 (C) [60.9 ± 5.5]	63.6 [44.4 ± 16.9]	14.81 ± 4.14
bital	1144.0 ± 318.6*	F (16:4)	18.7 (P) [33.3 ± 16.7]	83.3 [83.3 ± 16.7]	8.75 ± 2.27
Valproate	960.3 ± 155.4	M (17:4)	47.0 (C) [49.6 ± 9.1]	58.8 [58.8 ± 5.9]	11.88 ± 4.48
	1226.0 ± 160.6*	F (12:4)	33.0 (P) [34.3 ± 8.7]	75.0 [78.3 ± 11.7]	6.37 ± 1.32

*Significantly different from similar sex controls, $p<0.05$ or $p<0.01$.

†Locomotor activity was assessed only in animals injected with 0.9% NaCl; 5–6 offspring were used for each determination of SMA.

‡Spontaneous alternation data were first analyzed for position preference in order to determine the *a priori* probability of alternation for each treatment group. This value was then used in the Chi Square statistic to determine whether alternation behavior was significantly different from chance. Letters indicate Preserverative Behavior (P), Chance Levels (C), or Reliable Levels of Alternation (A). (Litter mean ± S.E.M. are listed in brackets.)

§Number of animals and litters in each determination is indicated in parentheses (animals:litters). Animals were tested for both alternation and cliff avoidance; order of testing was counterbalanced. (Litter mean ± S.E.M. are listed in brackets.)

TABLE 4

SERUM PROTEIN IN OFFSPRING PRENATALLY EXPOSED TO AEDs

Postnatal Age		Control		Phenobarbital		Valproate		Clonazepam	
		Albumin‡	A/G Ratio§	Albumin	A/G Ratio	Albumin	A/G Ratio	Albumin	A/G Ratio
Day 0–1	Male (12)¶	25.38±1.17	0.570±0.036	21.24±0.26 *	0.438±0.037 *	19.64±0.48 *	0.398±0.009 *	21.65±0.26 *	0.427±0.002 *
	Female (12)	20.48±1.68	0.428±0.040†	19.00±0.22	0.362±0.005†	19.42±0.25	0.372±0.005†	20.30±0.18	0.392±0.006†
Day 7	Male (12)	21.61±0.32	0.358±0.007	19.46±0.14	0.350±0.006	25.32±0.46 *	0.428±0.009 *	24.20±0.17 *	0.417±0.002 *
	Female (12)	22.21±0.68	0.401±0.124	20.90±0.23	0.376±0.012	21.72±0.51	0.368±0.008	22.92±0.35	0.430±0.004
Day 30	Male (12)	20.02±0.91	0.326±0.027	24.45±1.28	0.411±0.036	22.88±1.20	0.382±0.030	23.70±1.08	0.428±0.033
	Female (12)	22.54±0.77	0.384±0.018	24.40±0.81	0.421±0.021	23.54±1.45	0.411±0.034	24.57±1.39	0.426±0.037

All data are expressed as mean±standard error of the mean.

*Significantly different from controls, $p<0.05$ or $p<0.01$.

†Females significantly different from males, $p<0.05$ or $p<0.01$.

‡Expressed as a percent of total protein (Mean±S.E.M.).

§Albumin/Globulin (A/G) ratio was calculated by computing albumin and globulin fractions from the total protein electrophoresis scan.

¶Number in parentheses represents number of animals in each prenatal treatment group.

the animal chose. The results of this test are shown in Table 3. Differences among latency values for pups in each prenatal treatment group were not significant. However, latencies for all female offspring were significantly shorter than those for males, $F(1/75)=3.98$, $p<0.05$. The percent of offspring in each group exhibiting CA was analyzed by Chi Square. A significant difference among the groups was found for both males ($\chi^2(3)=11.41$, $p<0.01$) and females ($\chi^2(3)=13.09$, $p<0.01$). Additional analyses of these data using the litter, rather than the individual offspring, as the unit of analysis yielded similar results (Males: $\chi^2(3)=13.54$, $p<0.01$; Females: $\chi^2(3)=13.93$, $p<0.01$).

Serum protein in offspring. Analysis of serum protein levels at PND 0–1 indicated that the A/G ratio was significantly reduced in the offspring by prenatal AED exposure, $F(3/32)=11.28$, $p<0.01$. This reduction represented a decrease in the percent of albumin, $F(3/32)=5.83$, $p<0.01$, and an increase in the percent of globulins, $F(3/32)=5.03$, $p<0.01$. In control and phenobarbital offspring, the A/G ratio in females was significantly lower than in males; this difference was not seen in offspring exposed to valproate or clonazepam, $F(3/32)=3.02$, $p<0.05$.

At PND 7, the reverse occurred. The decrease in A/G ratio in phenobarbital offspring seen one week earlier was no longer apparent; differences were now observed between controls, valproate and clonazepam animals, $F(3/32)=12.74$,

$p<0.01$. The increase in the A/G ratio primarily reflected increases in percent albumin. At 30 days of age neither the serum A/G ratio nor its component protein fractions differed significantly as a result of prenatal AED exposure (Table 4).

DISCUSSION

The present results demonstrate that in most cases the offspring of females treated with AEDS during the last third of pregnancy were protected from PTZ-induced minimal and/or maximal seizures. This protection occurred in the absence of overly recognizable morphological abnormalities, and at drug doses which approximate those in routine clinical use. This behavioral change, however, was not a general drug effect but reflected an interaction between a specific AED and the sex of the offspring. Prenatal phenobarbital protected both male and female offspring from minimal seizures following 35 mg/kg of PTZ and maximal seizures following 50 mg/kg of PTZ. Prenatal valproate also afforded the offspring protection from minimal seizures. However, only female offspring were protected from maximal seizures; in males, the convulsive response to 50 mg/kg of PTZ was enhanced. Prenatal clonazepam exposure also protected both sexes from minimal PTZ seizures, and reduced the severity of maximal convulsions.

A decrease in PTZ-induced seizure mortality has been observed in 30-day old offspring following maternal exposure to the CNS depressant drug ethanol on GD 1–20 [6]. These authors speculate that this protective effect may be due to the increase in fetal brain levels of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) which has been reported following ethanol ingestion by pregnant rats [44]. This hypothesis is particularly appealing since PTZ is a GABA-specific antagonist in the CNS and part of its convulsant action is based on selective blockade of GABA-mediated inhibition [30]. Its possible relevance to the present data is suggested by the fact that the anticonvulsant action of valproate, the benzodiazepines and, to some extent, phenobarbital may be mediated in part via GABA-ergic mechanisms [24, 43, 48]. Additionally, the uptake of GABA is enhanced in the brain of young mice prenatally exposed to phenobarbital [36].

The behavioral alterations and learning deficits reported in previous studies have been consistent with the idea that prenatal AED exposure produces a generalized depression of CNS function. However, the effects on the non-convulsive behaviors sampled in the present study are not consistent with the hypothesis. The fact that changes in spontaneous motor activity and spontaneous alternation do not parallel alterations in convulsive behavior and sensory function was unaffected together with the sex-specific nature of the changes suggest that any effect on brain function might be limited to specific brain areas [16, 45, 47, 59, 62].

Alterations in seizure susceptibility could alternatively reflect changes in drug metabolism. Phenobarbital is the classic inducer of microsomal enzymes in liver and a recent report indicates *in utero* exposure to valproate increases neonatal hepatic enzyme activity [39]. Therefore, the seizure protection afforded animals prenatally exposed to these AEDs might result from increased degradation of PTZ rather than changes in CNS sensitivity. It is less feasible that metabolic changes account for the decrease in seizure susceptibility following prenatal exposure to clonazepam, since enzyme induction has not been reported for the benzodiazepines.

A third explanation is that prenatal AED exposure altered drug binding capacity and this accounted for the changes in seizure susceptibility. Although there were alterations observed during the first postnatal week, the lack of differences in any of the proteins evaluated at 30 days of age suggest that these changes were transient and that altered protein binding of PTZ is probably not responsible for changes in seizure susceptibility observed at 35–38 days of age.

Prenatal exposure to valproate and to clonazepam induced a sexually dimorphic response to PTZ convulsions which was unexpected in prepubertal animals. The enhanced responsiveness of the male offspring in these drug groups to PTZ when compared with their female siblings might reflect developmental alteration in hormone-responsive CNS substrates or perinatal hormone status. During gestation, the male fetus is more susceptible to insult than the female, because differentiation of the male brain requires the action of androgens at the appropriate developmental periods [19,64]. In addition, recent evidence indicates that prenatal exposure to centrally acting drugs can alter both reproductive and non-reproductive behavioral sex differences in the rat [15,31].

Our results demonstrate that doses of AEDs in the human therapeutic range which did not induce identifiable biochemical or anatomical changes in the brain can produce developmental deficits which alter the overall excitability of the CNS as evidenced by seizure susceptibility.

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