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NAME OF AUTHOR/NOM DE L'AUTEUR Wolfgang-J. PUSZTAY

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Increased Seizure Resistance in Rats Treated
Postnatally with Flurazepam

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Submitted by
Wolfgang J. Puszta

in partial fulfilment of the requirements for
the degree of
Master of Arts

.....
Thesis Supervisor

.....
Chairman, Department of Psychology

Carleton University

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Abstract

The long term effects on seizure susceptibility after neonatal exposure of rats to flurazepam was investigated.

Animals were tested for audiogenic seizure susceptibility throughout development and for sensitivity to pentylenetetrazol- and picrotoxin-kindled seizures during adulthood. The results indicate that a slight, but nonsignificant attenuation of acoustic startle responses was evident in neonatal flurazepam-exposed rats at 13 days of age. There was, however, no indication of severe audiogenic seizure susceptibility in either the control or neonatal flurazepam-treated rats. There was a significant reduction of body weights in the flurazepam treated rats at 10 days of age, but this was not found to persist.

The results for pentylenetetrazol and picrotoxin seizure kindling suggested an increased resistance to the development of more severe seizures in the neonatal flurazepam-treated rats, as evidenced by both lower frequency and reduced severity of seizures. An exception was found in the case of female rats kindled with picrotoxin where lethal effects were found at doses which did not induce seizures in males, so that a description of female kindling rates was not possible.

The findings were discussed in regards to future research which should ascertain the mechanisms involved in the neonatal flurazepam-induced seizure resistance.

Table of Contents

	page
Acknowledgements.....	iv
Abstract.....	iii
List of Abbreviations.....	v
Introduction.....	1
Experiment 1	
Introduction.....	11
Method.....	11
Results.....	17
Discussion.....	22
Experiment 2	
Introduction.....	26
Method.....	28
Results.....	33
Discussion.....	37
General Discussion.....	39
Suggestions for Future Research.....	43
References.....	45
Appendix.....	52
Table A.....	53
B.....	54
C.....	55
D.....	56
E.....	58
F.....	58
G.....	59
H.....	60
I.....	61
J.....	62

List of Abbreviations

AGS	--	audiogenic seizure
ASR	-	acoustic stimulation response
BZ	-	benzodiazepine
cm	-	centimetre
CNS	-	central nervous system
DA	-	dopamine
dB	-	decibel
DZP	-	diazepam
EEG	-	electroencephalogram
FZP	-	flurazepam
GABA	-	gamma-aminobutyric acid
h	-	hour
Hz	-	hertz
ip	-	intraperitoneal
kg	-	kilogram
mg	-	milligram
min	-	minutes
ml	-	millilitre
n	-	number per group
NE	-	norepinephrine
PTZ	-	pentylentetrazol
PXN	-	picrotoxin
sc	-	subcutaneous
sec	-	seconds
5-HT	-	serotonin
W	-	watt

Introduction

Benzodiazepine (BZ) compounds are among the most widely prescribed therapeutic agents for the treatment of anxiety, sleeplessness, muscle spasms and seizure disorders, although their mechanisms of action, long-term efficacy and margin of safety are controversial (Bellantuono, Reggi, Tognoni & Garattini, 1980; Greenblatt & Shader, 1978; Haefely, 1978; Smith, 1979). Recent identification of brain-specific BZ binding sites in humans and other vertebrates has provided new inroads for the investigation of their possible modes of action in the central nervous system (CNS) (Mohler & Okada, 1978; Squires & Braestrup, 1978). While the developing animal has provided a useful model in studying maturationally-linked responses to anticonvulsants and their modes of action (Vernadakis & Woodbury, 1969), examination of pre/postnatal effects of BZ exposure has generally focussed on possible teratogenic effects, a number of malformations being reported in lower animals and humans (Aarskog, 1975; Barlow, Knight & Sullivan, 1980; Buttar, 1980; Cree, Meyer & Hailey, 1973; Schiff, Chan & Stern, 1971; Shah, Donaldson & Burdett, 1979; Walker & Patterson, 1974). The possibility of long-term behavioral alterations following BZ exposure early in development has not, however, been extensively investigated.

Prolonged treatment of adult humans (Winokur, Rickels, Greenblatt, Snyder & Schatz, 1980) and rats (Sepinwall & Cook, 1978) with BZ's has been shown to produce tolerance to the pharmacological effects of these drugs. Furthermore, cessation of chronic BZ administration has been reported to result in a withdrawal syndrome, i.e. hyperexcitability, hyperreactivity, increased seizure susceptibility (Bantutova, Ovcharov & Koburova, 1978; Rastogi, LaPierre & Singhal, 1978; Winokur, et al., 1980). Swiss-Webster mice repeatedly exposed to a number of BZ's during pre- and/or postnatal development were found to be hyperactive when tested in a Y-maze apparatus during adulthood (Fox, Abendschein & Lachen, 1977). Exposure of fetal rats to diazepam (DZP) has also been reported to alter development of spontaneous locomotor activity while attenuating acoustic startle responses normally maturing at 2 weeks of age (Kellogg, Tervo, Ison & Parisi, 1980). Recently, in an investigation requiring neonatal exposure of rats to flurazepam (FZP, hydrochloride, Days 1-15) it was found that a small number of animals became extremely difficult to handle in adulthood, showing hyperresponsiveness to sudden movements and noise (Pusztay, unpublished observations). Spontaneous convulsive behavior was also observed, as related to the experimenter by the vivarium staff. While a

controlled examination of this phenomenon was not possible at the time, a number of animals were subsequently screened for audiogenic seizure (AGS) susceptibility employing a similar procedure as described by Jobe, Picchioni and Chin (1973a). The results of these tests suggested possible increased AGS susceptibility in neonatal FZP-exposed rats (as indicated by the incidence and intensity of seizures), although strain-specific or genetic predisposition could not be ruled out as possible mediating factors. A summary of these preliminary findings is included in Table A of the Appendix.

Consequently, a second experiment was conducted employing a reduced drug regimen (5mg/kg FZP; Days 1,3,5,7,9) administered to off-spring of Wistar rats bred in our vivarium. After completion of AGS testing on Days 16,18,20,22 and 24, it was found that 4 out of 10 neonatal FZP-exposed rats and 2 of 10 control animals evidenced seizure susceptibility, as judged by the ratings given for their responses during acoustic stimulation. However, 1 of 2 AGS susceptible rats from the control group demonstrated maximal convulsive scores (tonic/clonic) by Day 24, whereas the maximum response recorded for neonatal FZP-exposed rats was only wild running. Jobe and associates (1973a) have suggested that wild running (sudden, explosive and aimless)

itself should not be considered a convulsive episode, but more so a precursor to actual seizure responses of evidence of susceptibility which can result from multiple exposures to intense acoustic stimulation in young rats (Brown, Jobe, Bairnsfather, Mims & Woods, 1980).

These results seemed to suggest that neonatal FZP exposure could be responsible for alterations in the effects of multiple acoustic stimulations in young rats, producing perhaps an earlier occurrence or greater frequency of AGS susceptibility which could possibly lead to the development of more intense convulsive episodes later in life. It was therefore decided that a more extensive analysis of neonatal FZP exposure and its possible role in modulation of AGS in the developing rat was warranted.

AGS Susceptibility in Rodents

In humans, responses to antiepileptic drugs vary with the severity and type of epileptic seizure. While animal models employing AGS, electroshock and convulsogenic drugs are common procedures for evaluating novel anticonvulsant drugs, neither one alone is capable of identifying all known clinically effective antiepileptics and they are inconsistent in regards to interpreting epileptic specificity and discrete mechanisms of drug action(s) (Krall, Pendry, White, Kupferberg & Swinyard, 1978;

Woodbury, 1972). More recently however, it has been suggested that the AGS-prone rat may be a model for not only the rarely occurring audiogenic epilepsy, but may also serve as a genetically based model for the more common generalized and/or partial epilepsies (Consroe, Picchioni & Chin, 1979; Consroe, Kudroy & Schmitz, 1980).

Natural susceptibility of rodents to sound-induced seizure activity has been recognized and extensively researched by numerous investigators in various fields (for reviews see Bevan, 1955; Consroe, Picchioni & Chin, 1979). Genetic disturbances, infectious disease, trauma, postnatal insult, circulatory, metabolic and nutritional upset, to name but a few acquired conditions, have all been implicated in the etiology of AGS susceptibility. Since only about 20-50% of rodent populations normally evidence AGS, selective breeding of animals has been used to increase the incidence and uniformity of the response. Furthermore, seizure responses to electroshock and some convulsant drugs have been found to increase, whereas the anticonvulsant effects of some antiepileptic drugs decrease with the progression from AGS-resistant to AGS-prone rat strains. This suggests to some researchers that the AGS-prone rat model may provide important pharmacogenetic, aetiological and pathophysiological information that relate to human

epilepsies and are not readily apparent with the more traditional electroshock and chemical convulsant models (Consroe et al., 1979).

As mentioned, descriptive classification of AGS activity is uniform in susceptible rodents. Intense AGS activity proceeds from initial acoustic startle to quiescence, followed by episodes of wild running, and in more extreme cases leads to tonic/clonic movements of the body and limbs often resulting in death or at best recovery (Jobe, et al., 1973a). Expression of AGS responses has been demonstrated to occur early in development (between 13 and 31 days of age), while susceptibility to AGS has been found to decrease with age, being virtually non-existent in adult rodents (Finger, 1943).

Neurochemical disturbances involving norepinephrine (NE), dopamine (DA), serotonin (5-HT) and gamma-aminobutyric acid (GABA) have all been implicated in AGS (Jobe, et al., 1973 a,b; Jerlicz, Kostowski, Bidzinski, Hauptman & Dymecki, 1978; Yanai, Sze & Ginsburg, 1975) in addition to pituitary-adrenocortical activity (Borrell, Torrellas, Guaza & Borrell, 1980). Unfortunately, there is no unifying theory available which might explain the involvement of neurotransmitters in AGS (Consroe, et al., 1979). Reserpine-induced reductions of NE, DA and 5-HT have been

shown to reduce thresholds for electrically and chemically-induced seizures (Chen, Ensor & Bohner, 1954; London & Buterbaugh, 1978) while increasing the intensity of AGS (Lehmann, 1970). Selective depletions of cortical and spinal NE following intracerebral injection of 6-hydroxydopamine (Bourn, Chin & Picchioni, 1972) or 5-HT depletions (Jobe, et al., 1973b) have also been demonstrated to increase AGS severity. It is now suggested that at least some of the effects of sound stimulation and AGS severity might be mediated by brain NE pathways in conjunction with adrenal-pituitary activity (Borrell, et al., 1980). Still other investigators have demonstrated that disruption of glutamate or GABA activity results in increased AGS susceptibility, while injections of these amino acids in AGS-prone rats blocks the convulsogenic effects of sound stimulation (Consroe, et al., 1979; Lehmann, 1970).

Neuroanatomical and neurophysiological investigations have suggested a primary role for selective auditory (cochlear nuclei, inferior colliculi, medial geniculate bodies) as well as non-auditory subcortical sites (vestibular nuclei, midbrain reticular formation, septal nuclei) in AGS (Shnerson & Willott, 1979). Defects in auditory systems or neuronal sites outside primary auditory pathways have been shown to enhance AGS susceptibility.

(Tepper & Schlesinger, 1980), although cortical influences cannot be discounted, since spreading of seizures throughout the cerebral cortex has long been considered a basic mechanism in epilepsy (Bourn, et al., 1977).

Experiential factors such as housing conditions, stress, and in particular, early sound stimulation can also modulate AGS susceptibility (Bevan, 1955; Brown, et al., 1980; Henry, 1967). Acoustic priming, or brief exposure of resistant strains of mice to the intense ringing of an electric bell at 16 days of age will result in severe AGS when tested again at 21 days (Henry, 1967). Similarly, Brown and associates (1980) demonstrated that normally-resistant strains of rats will also develop AGS susceptibility when exposed to multiple sound stimulations between the ages of 13 and 31 days. Unlike results for AGS-prone rats, however, changes in the intensity of responses do not increase beyond the initial stage of wild running.

The effects of acoustic stimulation on AGS in young rats and mice has been suggested to be an instance of disuse-supersensitivity; cochlear damage due to intense auditory stimulation during a critical period of development results in a reduction of afferent input to neuronal structures, such that subsequent reexposure to intense

stimulation results in a massive volley originating from the still intact cochlear elements, which in turn triggers a convulsive response (Saunders, Bock, James & Chen, 1979; Tepper & Schlesinger, 1980). Such a claim has not been substantiated. Considering that AGS development in resistant strains of rats seemingly requires intense auditory stimulation during a critical period of cochlear maturation (Shnerson & Willott, 1979), it might be expected that early exposure of rats to a BZ might attenuate, or at least delay AGS onset, since Kellogg and associates (1980) did find that prenatal exposure of rats to diazepam (DZP) resulted in the attenuation of acoustic startle responses normally maturing at 2 weeks of age. Preliminary results using postnatal FZP exposure (see Table A, Appendix) suggested, however, that AGS susceptibility is at best unaffected if not enhanced. Prolongation of a critical period for AGS development could possibly be due to BZ withdrawal, manifested in part by a transient seizure susceptibility as noted in adult rats (Bantutova, et al., 1978; Rastogi, et al., 1978). Evidence of increased AGS susceptibility later in development or even adulthood would then suggest a more long-lasting alteration, possibly due to CNS functional alterations resulting from postnatal BZ exposure.

Alternatively, undernutrition resulting from inadequate suckling behavior due to over-sedation with a BZ could perhaps provide a simpler explanation for any long-term effects of the drug. Underfeeding during the suckling period has been shown to produce abnormal reactivity and emotionality in rats (Villescas, Zamenhof & Guthrie, 1979).

Experiment 1

The purpose of the present investigation was to provide a developmental analysis of AGS in rats exposed to the benzodiazepine, FZP, in infancy. A comparison of AGS susceptibility in animals tested at different age intervals following cessation of drug administrations permitted an assessment for transient or perhaps permanent effects from such early drug exposure. Furthermore, the possible relationship of undernutrition of animals due to chronic FZP exposure and effects on the development of AGS in rats was assessed through correlational analysis of individual body weights and behavioral seizure activity.

Method

Subjects Off-spring of 20 Sprague-Dawley pregnant female rats (timed pregnancies; obtained at 15 days gestation), purchased from Canadian Breeding Farms (Montreal) were born and housed in maternity cages located in the Department of Psychology Vivarium. All litters born within a 12 h time period were simultaneously removed from their respective dams, sexed, and randomly distributed to litter groups of equal numbers of males and females ($n=12$). The litters were then randomly reassigned to a dam for fostering, followed by random allocation of actual treatments to groups, comprising

6 factorial combinations to be specified. The 12 remaining litters born were also fostered at this time and then assigned for neonatal treatments in preparation for subsequent investigations in adulthood.

Lighting was maintained on a 12 h light/12 h dark cycle (lights on at 0800 h and off at 2000 h) throughout the investigations. Group housing conditions were also maintained during infancy and throughout the post-weaning period by transferring litters from maternity to colony cages, housing either 6 males/females.

Neonatal Drug Treatments - For all experiments, rat pups assigned to the drug condition received subcutaneous injections of FZP (5.0 mg/ml, Dalmane, Roche) in proportion to individual body weights, yielding an estimated dose of 5.0 mg/kg on Days 2, 4, 6, 8 and 10 of life. This drug and parameters were chosen with consideration for the water solubility of FZP hydrochloride and the inability to obtain the more widely used drug, diazepam, with similar solubility. Furthermore, this drug, dose and route of administration was found to be effective in eliciting significant changes in the activity of neonatal rats, as well as demonstrating potent anxiolytic effects in adult rats in a conflict situation (personal observations). The oral route of administration was avoided simply because of

the difficulty of gavaging such young rats. Comparable injections of saline solution were administered to control animals on the days specified. In total, 9 litters (n=12) received neonatal FZP injections, 3 of which were used in the present investigation.

AGS Testing Apparatus - The testing chamber consisted of a clear plexiglass box (45 x 30 x 40 mm) which was halved by a black divider, and located in a sound-attenuating plywood chamber (58 x 45 x 61.75 cm) insulated with acoustic ceiling tiles and lined with aluminum foil. The front door of the plywood chamber was locked during testing, while an observation window (19 x 19 cm) provided for unobstructed and continuous visual monitoring of animals during testing. The interior of the chamber was lighted by a 7.5 W bulb, while fresh air was circulated by fans housed within the walls of the wooden chamber.

An electric bell (109 dB, 60 Hz; Adaptabell, Edwards Co.) 10.5 mm in diameter was mounted in the center of the interior ceiling of the wooden chamber. The duration of the sound stimulus was controlled by interval timers and set for 110 sec, to be initiated manually by the experimenter.

AGS Testing Procedure - Each of the 3 FZP and 3 control group litters had been assigned to one of three AGS testing

ages; either Days 13-31, 26-44 or 39-57, with testing beginning on the first day and repeating every 3rd day for a total of 7 trials. Selection of the stimulation intervals and earliest age for testing was based on the recommendations of Dr. P.C. Jobe (personal communication), who has conducted several such studies in the rat (Jobe, et al., 1973a,b; Brown, et al., 1980).

On the first day of testing, all animals were transported to the testing area, housed in their respective home cages located on a single rack. The animals' body weights were recorded, and their toenails were clipped for identification purposes. When ready, one experimental and one control animal were simultaneously placed in either side of the plexiglass chamber, their underbellies first being marked, thereby permitting recognition of group membership upon completion of a trial, and allowing some degree of 'blind' assessment of responses during actual testing. After the animals were in position and the door was locked, a 20 sec habituation period was allowed before onset of the sound stimulus. Following continuous observation of the animals during the intense sound stimulation to yield a rating of their maximal response, animals were removed and quietly replaced in their home cages.

AGS Scoring Procedure - Animals were assigned scores on the basis of the following acoustic stimulation response (ASR) scale, adapted from Jobe and associates (1973a) and described in Table 1.

Table 1

ASR ratings used in assigning scores to animals during AGS testing throughout development

ASR Rating	Behavior
1	startle response, hypermotility
2	startle response followed by a continuous episode of wild running for the duration of acoustic stimulation
3	startle response followed by temporary wild running, quiescence, and then a return to wild running
4	startle response, temporary bout of wild running, proceeding to tonic/clonic movements of the torso and limbs

For statistical analysis of group differences in ASR, the numbers of trials to the first occurrence of successive rankings of ASR were used as dependent variables as a measure of seizure-proneness. The number of ASR rankings used in the statistical analyses was dependent upon the behavioral seizures evidenced by animals during the 7 trials, since discriminating measures were unknown prior to experimentation, thereby necessitating a multivariate approach. For those animals not reaching a particular ASR

rating evidenced by others during 7 trials, a score of 8 (7 plus one additional trial) was arbitrarily recorded for that ranking. For example, if an animal only expressed wild running (ASR=2) as a maximal response during testing, where others evidenced two bouts of wild running (ASR=3), a score of 8 was recorded for the (unachieved) ASR=3 rating.

Hotellings T square was then used to examine the mean number of trials to occurrence of startle responses (ASR=1), a single episode of wild running (ASR=2), and 2 bouts of wild running (ASR=3). Testing for significant differences between groups was conducted only for the youngest groups (Days 13-31), since only these rats evidenced signs of seizure susceptibility.

The two drug groups were also compared for statistical differences in body weight at 10, 13, 26 and 39 days of age in order to assess the possible effects of FZP exposure on nutritional status, as indicated by body weights.

A regression analysis of the possible relationship between body weights and ASR ratings as they developed during acoustic stimulations was conducted in the 13 to 31 day old rats. Older age groups were not statistically examined in this manner, since the results did not show any variability in the seizure resistance of animals.

Results

Effects of FZP and Sex of Animals on Body Weights - Neonatal

FZP exposure was found to result in significantly lower body weights of 10 day old rats, regardless of their sex, $F(1,212) = 26.12$, $p < .001$. In addition, female rats were found to be significantly lighter than males, $F(1,212) = 7.89$, $p < .005$, an effect which seemed accentuated in neonatal FZP-exposed females, but not confirmed by a significant interaction, $F(1,212) = 0.44$, $p < .51$.

ANOVA of mean weight differences of animals tested for AGS beginning on Days 13, 26 and 39 did not, however, indicate any significant effects of the neonatal FZP treatments, while sex and age of the animals did, as expected, have effects as evidenced by a significant sex by age interaction, $F(2,60) = 36.07$, $p < .001$. At 39 days of age, males were significantly heavier than their female counterparts. A summary of mean body weights for all animals examined is provided in Table 2.

Table 2

Effects of neonatal FZP exposure on the body weights of Sprague-Dawley rats at various ages in development. Standard errors are in brackets

Group	n	Age (days)	Mean Weights (g)
Control			
Males	57	10	19.83 (.41)
	6	13	19.11 (1.02)
	6	26	65.47 (2.08)
	7	39	151.50 (3.51)
Females	51	10	19.02 (.35)
	6	13	22.28 (.83)
	6	26	64.58 (1.33)
	5	39	123.12 (4.44)
Flurazepam			
Males	54	10	18.15 (.37)
	6	13	20.06 (.50)
	6	26	62.68 (1.21)
	6	39	156.33 (3.68)
Females	54	10	16.83 (.39)
	6	13	19.18 (.81)
	6	26	56.77 (3.93)
	6	39	119.83 (4.68)

Correlation of Body Weights with ASR Rating - No significant relationship was found between individual body weights on Day 13 (the first day of AGS testing) and the animals' scores during AGS testing. Table 3 provides a summary of the raw regression coefficients, t-values and significance levels.

Table 3

Relationship between individual body weights of rats and the number of acoustic stimulations required to elicit ASR ratings during AGS testing on Days 13 to 31. Standard errors are in brackets

ASR	Raw Regression Coefficients	t	p<
Acoustic Startle Response (1)	-6.093 (.04)	-1.54	.14
Wild Running One Bout (2)	-2.184 (.10)	-.23	.82
Wild Running Two Bouts (3)	-2.683 (.10)	-.26	.80

Effects of FZP on the Development of AGS - No significant

differences between groups were noted in the number of trials to the first occurrences of the 3 ASR's observed (startle responses, wild running, two bouts of wild running), $T \text{ square} = 7.56$, $p < .11$. Exploratory univariate analyses did, however, suggest that a slight, nonsignificant reduction in startle response frequency was evidenced in neonatal FZP-exposed rats when examined on Day 13, $F(1,22) = 1.77$, $p < .09$ (see Table B, appendix, for raw data). No statistical analyses of FZP-induced differences in ASR were conducted for the groups for which testing was initiated at 26 and 39 days of age, since all the animals showed only startle responses. A summary of the number of

animals attaining the various categories of ASR during the 7 trials is provided in Table 4; the raw score ASR ratings actually recorded for groups for each trial are provided in Table B of the Appendix.

Table 4

Effects of neonatal FZP exposure on ASR during 7 trials between the ages of 13 and 31, 26 and 44, 39 and 57 days.

Group	Age at Testing	n	No. Animals Expressing ASR*		
			1	2	3
Control	13 - 31	12	9	1	2
	26 - 44	12	12	nil	nil
	39 - 57	12	12	nil	nil
FZP	13 - 31	12	11	1	nil
	26 - 44	12	12	nil	nil
	39 - 57	12	12	nil	nil

* 1 - startle response; 2 - wild running; 3 - two bouts wild running

Table 5 provides the summary statistics for the analyses of group differences in mean number of trials to the first occurrence of successive ASR ratings used for animals tested between 13 and 31 days of age.

Table 5

Effects of neonatal FZP exposure on AGS development as indicated by the mean number of trials to the first occurrence of successive ASR ratings recorded during testing between 13 and 31 days of age. The standard errors are shown in brackets.

Group	n	Acoustic Startles	Wild Running	Two Bouts Wild Running
Control	12	1.16 (.11)	7.42 (.36)	8.33 (.47)
FZP	12	1.50 (.15)	7.75 (.25)	9.00 (.00)

Discussion

Contrary to what was expected, subacute neonatal FZP exposure did not result in a significant increase in the development of AGS in rats, under the conditions used in this study. In retrospect, these findings are similar to those of the previous pilot work which employed a drug regimen which was chosen not to interfere with AGS testing at 2 weeks of age, AGS testing having begun on Day 16 and repeated every second day until 24 days of age. In both studies control animals experienced more severe seizures as compared to bouts of wild running in FZP-exposed rats (see Appendix, Table A). The initial finding of seizure susceptibility in older animals, first noted by vivarium staff, cannot be readily explained or supported by these investigations. The AGS testing procedure in this experiment was selected after the recommendations of Dr. P.C. Jobe (personal communication), who with his coworkers has recently demonstrated AGS priming in rats using ages and testing intervals similar to those employed here (Brown, et al., 1980). There are obvious differences between the incidence of AGS for the control animals in the present experiment and that reported by Brown and associates (1980) using similar strains of resistant rats. Their results indicated about .50 greater probability of occurrence of

wild running by 31 days of age than was indicated in the present investigation (15 of 36 rats as compared to the 3 of 12 reported here). There may be some critical differences between that study and the present one. For example, Brown and associates used two electric bells for acoustic stimulation; the present study used only one. Differences in housing (one rat per cage versus group housing throughout in the present study). Handling practices and the hour of the day that the animals were tested could also have affected the results. Perhaps then, weaknesses in the AGS procedures employed here reduced the chances of detecting significant differences.

The significant lowering, in neonatal FZP-exposed rats, of body weights in males and females on Day 10, suggests that factors related to the nutritional requirements of pups during the suckling period may have been affected by the drug. This effect on body weights was not, however, found to persist beyond this age, nor was it found to be related to AGS susceptibility. Worthy of mention was the incidental observation that none of the FZP-exposed pups evidenced eyelid opening by 13 days of age, in contrast to the control animals where 7 of 12 rats had at least one eyelid completely open.

The tendency towards less frequent acoustic startle

responses in neonatal FZP-exposed rats at 13 days of age parallels the results of Kellogg and associates (1980) who found significant attenuation of acoustic startle responses in 2 week old rats prenatally-exposed to DZP, a more potent anxiolytic/anticonvulsant than FZP (Straughan, 1979; Sepinwall & Cook, 1980). It might be that the development of acoustic startle responses during the postnatal period was only weakly affected by FZP, whereas more prominent effects might be evidenced using the more potent benzodiazepine, DZP.

In summary, the present observations suggest that neonatal FZP exposure does effect delayed maturation of rats, as evidenced by the lack of eyelid openings at 13 days of age, and lower body weights at 10 days. There were, however, no obvious effects on the development of seizure susceptibility, judging by AGS testing and apart from the slight attenuation of acoustic startle responses on Day 13. Furthermore, these results suggest that the drug regimen used does not seemingly produce withdrawal responses in animals (perhaps due to the low drug dose and/or the short duration of treatment), since increased seizure susceptibility at one of the age intervals tested for AGS would have suggested withdrawal reactions, but was not observed.

Because of the discrepancy between AGS susceptibility in the strain of rats used here and that reported for a similar strain by Brown and associates (1980), further research on the long-term effects of neonatal BZ exposure using this dependent variable (acoustic stimulation-induced seizures) seemed unwarranted. As mentioned above, various seizure models are employed since not one is capable of accurately identifying all clinically relevant antiepileptics or recognized seizure types, suggesting that any limitations of the AGS model used in the present study may be due to specific characteristics of that disorder which may not be generalizable to seizure susceptibility per se; in particular its reliance on early postnatal acoustic experiences and heredity (Consroe et al., 1979). Therefore it was decided to assess seizure susceptibility of neonatal FZP-treated rats using more traditional convulsogenic procedures shown to be effective in adult animals, thereby providing a more thorough examination of the potential for motor seizures.

Experiment 2

There has been a report of enhanced susceptibility to the chemical convulsant pentylenetetrazol (PTZ) in adult rats chronically exposed to and withdrawn from a BZ (Bantutova, et al., 1979). Furthermore, prior exposure to convulsive agents, or simply pathogenic liability, has been demonstrated to exacerbate the effects of kindling (the progressive intensification of seizure activity which occurs during a series of low-intensity electrical brain stimulations or sub-threshold doses of chemical convulsants) and the effects of alcohol withdrawal (for reviews see Cain, 1980; McIntyre, 1980; Pinel, 1980; Racine, 1978). It has also been suggested that kindling procedures that do not in fact evoke afterdischarge or convulsions will nevertheless reduce the convulsive threshold and increase the likelihood of a convulsion by the same and other convulsive agents (Cain, 1980). Examination of the incidence of convulsions following chemical convulsant treatments in adult rats previously exposed to AGS testing procedures and/or FZP, which did not lead to a significant occurrence of motor seizures, could then be used to assess the pathogenic liability impaired by such early experiences and their more general effects on seizure susceptibility. In the following experiment this was studied with the drugs PTZ and

picROTOXIN (PXN).

Although the mechanisms of action for PTZ-induced seizures are still largely unknown, it is generally agreed to involve effects on both inhibitory and excitatory systems (Vernadakis & Woodbury, 1969; Woodbury, 1980), and that the altered relationship between different neurotransmitters, rather than a specific neurotransmitting system, may play a decisive role in its convulsant effects (Lazarova, Przewlocka, Mogilnicka & Stala, 1979). In laboratory rats, threshold convulsive doses of PTZ produce activation of the EEG and motor activity characterized by facial and forelimb clonus, while slightly higher doses result in generalized clonic movements and tonic extension with loss of postural equilibrium (Cain, 1980; Woodbury, 1980). Repeated injections of subthreshold doses of PTZ in rats have been demonstrated to induce myoclonic responses following a small number of injections, developing into grand mal seizures by the end of the experiment (Pinel, 1980).

The mechanisms of action for PXN-induced seizures are considered to involve a primary inhibition of postsynaptic increases in membrane chloride conductance, as triggered by GABA. PXN exerts a more or less selective blockade of GABA inhibitory transmission without actual antagonism of GABA receptor binding (Karobath, Drexler & Supavilai, 1981;

Olsen, Lee-Lundberg & Napias, 1980). In addition to effects on synapses, PXN, like PTZ, appears to have some direct excitatory effects on neuronal membranes, although the precise nature of these effects has not been studied in vertebrates (Woodbury, 1980).

The convulsions induced by PXN resemble closely the pattern produced by PTZ, where large doses result in generalized tonic/clonic convulsions, although a longer latency for seizures is evident (Duka, Holtt & Hertz, 1979). Kindling procedures in rats using subconvulsive doses of PXN results in a significant decrease of the seizure threshold after approximately 11 injections (Izquierdo, Oliveira & Settineri, 1975). Evaluation of kindling rates in neonatal FZP-exposed and saline control rats to PTZ and PXN-induced seizure activity, it was expected, would establish whether or not early drug experiences and/or AGS testing procedures have long-term effects on seizure disorders precipitated by dissimilar convulsive agents.

Method

Subjects - Sprague-Dawley rats either exposed to neonatal FZP or saline treatments and tested for AGS between the ages of 13-31 and 26-44 were included with other rats treated similarly in infancy, but who had not been tested for AGS. Instead, the latter had been simply placed in the AGS

apparatus without receiving acoustic stimulation for the time and ages specified. In all there were 71 animals comprising 4 treatment combinations that were available for kindling, using either PTZ or PXN.

Housing and lighting conditions were maintained in accordance with the specifications in Experiment 1.

PTZ Kindling - A dose of 50 mg/ml/kg pentylene-tetrazol (Sigma) dissolved in saline and administered ip was used to kindle 12 neonatal FZP-exposed rats, 6 of which had also been acoustically stimulated on Days 26-44, in addition to 12 comparable neonatal saline-treated animals. The dose used was suggested to be well below threshold for induction of clonic convulsions (Casrilevitz, Engelhardt & Esberard, 1971; Oishi, Suenaga & Fukuda, 1979; Soubrie, Thiebot, Jobert, Montastruc, Hery & Hamon, 1980). This suggestion was subsequently confirmed by pilot work with naive male animals where only myoclonic jerks occurred during a 15 min observation.

The testing procedure involved the weighing and injection of animals every second day beginning at 65 and ending at 73 days of age, for a total of 5 trials. Following injections, between 1600 and 2200 h, the rats were immediately placed in the plexiglass box also used in Experiment 1, which was now located in another laboratory.

Each animal was then observed for a period of 15 min, during which time the maximal seizure response was recorded, according to a rating scale adapted from Casrilevitz and associates (1971). A summary of response measures and ratings used is included in Table 5.

The total number of injections to the first occurrences of myoclonic seizures and clonic convulsions (ratings 3 and 4) were used for statistical analyses of group differences in seizure susceptibility using multivariate ANOVA (Finn & Mattsson, 1978). As was the case in Experiment 1, if an animal did not reach a particular stage of seizure (or seizure rating) as observed in others during testing a score of 6 (5 plus 1 additional trial) was arbitrarily recorded for the single unobtained rating. Similarly, if an animal failed to reach two successive ratings demonstrated in others; a score of 6 (5 plus 1 additional trial) was awarded for the lowest unachieved rating, and 7 for the next seizure ratings. For example, an animal having a maximal seizure response rating of 1 while others reached ratings of 3, resulted in the recording of a score of 6 for the missing ratings of 2, and 7 for the missing ratings of 3.

Table 6

Response measures and ratings used during PTZ and PXN
kindling procedures in neonatal FZP-exposed rats

Rating	Behavior
1	hypermotility and/or sniffing followed by slight increases in activity
2	gradual head retraction and tremors of the head and face
3	sudden head retraction, body torsion, clonic forelimb movements, loss of equilibrium
4	all of the above, followed by a sudden burst of wild running followed by tonic/clonic seizure
5	as above, followed by catalepsy and/or death

PXN kindling - The dose of 3.5 mg/ml/kg picrotoxin (Sigma) was administered ip. It had been selected as a subconvulsive dose for kindling, based on the findings of Vernadakis and Woodbury (1969) and pilot work with naive male rats. The 24 animals either exposed to FZP or saline injections postnatally, and tested for AGS between the ages of 13-31 were compared with 23 comparable neonatally treated rats who were placed in the AGS apparatus, but not acoustically stimulated. About half of the 47 animals were tested as a squad between the ages of 104 and 126 days of

age, and the remainder on Days 119-131.

As was the case for PTZ kindling, PXN was injected every second day during the ages and times specified, but was continued for a total of 10 trials. A similar testing procedure involving injection and observation was used, although animals were placed in the apparatus 15 min prior to actual observation and scoring of responses in order to accommodate the much longer onset of PXN effects (Soubrie, et al., 1980). Since PXN-induced seizure responses are similar to those induced by PTZ, the same rating scale was utilized, as outlined in Table 6.

Because of the unexpected lethal effects of PXN injections in female rats, the total number of injections to a rating of 4 and 5 were used for statistical examination of group differences in PXN seizure susceptibility using MANOVA. For the male rats, the total number of injections to the first occurrence of seizure ratings of 2 and 3 were used, and again, if any animal failed to reach either or both criterion during the 10 trials an arbitrary score (+1's or +2's) was recorded for each dependent measure, as previously described.

Results

PTZ Kindling - As shown in Table C of the Appendix, the initial injection of rats with 50 mg/kg PTZ induced mild seizure activity (rating = 3). MANOVA of group differences in the number of trials to the first occurrence of ratings = 3 and 4 indicated a significant effect of neonatal FZP exposure in delaying the development or limiting the severity of seizures, $F(2,19) = 4.51$, $p < .025$. The FZP-exposed rats required significantly more PTZ trials before the occurrence of generalized clonic convulsions (rating = 4) than neonatal saline controls (univariate $F(1,20) = 9.0$, $p < .007$). Interestingly, there were no significant interactions or main effects due to the AGS testing procedure. Table 7 provides the summary statistics for PTZ kindling. Testing was halted after 5 trials since about 83% of the animals had achieved ratings = 4.

PXN Kindling - All female rats undergoing PXN kindling died by the eighth trial, whereas no male died during the entire ten trial procedure. Since severe seizure activity (rating = 4) was evidenced in female rats by the first or second trial (see Table D, Appendix) it was impossible to compare kindling rates between the four treatment groups. The males, on the other hand, did evidence PXN kindling. Initial exposure to PXN did not induce convulsions, but

subsequent injections resulted in mild (myoclonic) seizure activity (see Table D of the Appendix).

Table 7

Effects of neonatal FZP exposure on subsequent kindling of PTZ-induced convulsions. Results using the mean (S.E.) number of trials to criterion*

Group	n	No. of Trials to Rating: *	
		3	4
Control			
AGS	6	1.00 (.00)	3.33 (.80)
NoAGS	6	1.50 (.34)	3.50 (.62)
Flurazepam			
AGS	6	1.33 (.33)	4.83 (.31)
NoAGS	6	1.00 (.00)	5.33 (.32)

* 3 - myoclonic seizure 4 - generalized convulsion

Statistical analyses of the number of trials to the first occurrence of clonic convulsions (rating = 4) and lethality (rating = 5) between neonatal FZP and saline-injected female rats showed no effect due to early postnatal treatments (See Table 8).

Table 8

Effects of neonatal FZP exposure on the development of increased seizure susceptibility induced by repeated administrations of PXN in female rats. Standard errors are in brackets

Group	n	No. of Trials to Rating	
		4	5
Control			
AGS	6	1.50 (.22)	4.83 (.87)
NoAGS	6	1.33 (.33)	4.50 (.43)
Flurazepam			
AGS	6	1.00 (.00)	5.83 (.70)
NoAGS	6	1.17 (.17)	4.67 (1.05)

4 - generalized convulsion 5 - death

MANOVA of differences between comparable male rats indicated no significant effect due to neonatal FZP exposure (see Table 9 for the summary statistics). Post hoc inspection of the numbers of males actually displaying myoclonic seizures by 10 trials (one of 12 FZP-exposed and 7 of 11 controls - see Table D of the Appendix) suggested that lack of statistically significant differences between groups may have been partially due to the arbitrary nature of scoring the number of trials to seizure criteria.

Table 9

Effects of neonatal FZP exposure on the development of PXN kindled seizures in male rats. Standard errors are in brackets.

Group	n	No. of Trials to Rating	
		2	3
Control			
AGS	6	7.17 (1.38)	9.67 (1.28)
NoAGS	5	4.80 (.49)	9.40 (.40)
Flurazepam			
AGS	6	8.17 (1.30)	11.50 (.22)
NoAGS	6	9.17 (1.32)	10.67 (1.15)

* 2 - myoclonic jerks 3 - myoclonic seizures

It was decided that a post hoc exploratory nonparametric analysis of group differences using the Kruskal-Wallis Test (Kirk, 1968) could provide an alternative method of assessing the results, employing a rank transformation of the number of trials to ratings of 2 and 3. Such a procedure is suggested for data which are badly skewed, as was the case in the present analysis. The results of this test were significant, $H=5.63$, $p<.018$, as indicated by the lower rankings associated with the fewer number of trials required to elicit myoclonic seizures in control groups (see Table 10).

Table 10

Results of exploratory nonparametric analysis of differences between neonatal FZP and saline-treated rats using the Kruskal-Wallis Test

Group	n	Rank Sums For Seizure Ratings	
		2	3
Control	11	102.5	95.0
FZP	12	173.5	181.0*

* significant, $p < .0176$

Discussion

The initial administration of PTZ was found to induce myoclonic activity in all rats, thereby disqualifying an analysis of a kindling phenomenon in the strictest sense. The results did, however, indicate a delay in the development of more severe seizure activity with subsequent PTZ injections in neonatal FZP-exposed rats. Considering that such an effect was evidenced in adulthood, it seems that a relatively long-term resistance to motor seizure activity was instated by neonatal FZP exposure. The possibility of increased convulsive liability following AGS testing and effects on PTZ-induced convulsions was not supported.

Of the 12 neonatal FZP-exposed male rats only one

showed any myoclonic activity as compared to 7 out of 11 neonatal saline treated rats during PXN kindling procedures suggesting similar, although not statistically significant results as was observed with PTZ.

Furthermore, at the dose used, PXN induced seizures with greater frequency and severity in female rats, irrespective of the neonatal treatment conditions, causing death after at most 8 administrations. It is noteworthy that sex differences were not observed during PTZ kindling, although it is possible that the dose used was too high, as expressed by the equipotent convulsive effects in both sexes after one administration. While sex differences to the toxicity of drugs are well established for rats with females generally displaying greater sensitivity (H. Buttar, personal communication, July, 1981), the present findings suggest differences specific to drug absorption/disposition and metabolism of PXN as compared to PTZ (for a review see Woodbury, 1980) in female rats.

General Discussion

The present investigations have provided indications of enhanced seizure resistance in neonatal FZP-exposed rats. Although no significant differences were evident in the AGS test during infancy, the retardation of startle responses (which are necessary for the development of AGS) on Day 13 approached significance in neonatal FZP-exposed animals. Neonatal FZP exposure caused a significant weight reduction at 10 days of age (although weight loss was not found to be related to measures of AGS susceptibility when tested between 13 and 31 days of age), as well as a delay in eyelid opening. The slight delay in acoustic startle responses is reminiscent of the more obvious delay found in prenatally-DZP-exposed rats (Kellogg et al., 1980).

There was a significant delay in the appearance of more severe seizure activity resulting from repeated injections of subconvulsive doses of PTZ in both male and female FZP-treated rats. No significant results were obtained during PXN kindling, although there was a suggestion of a similar delay in males. Females appeared however, to be hyperresponsive to the motor seizure and toxic effects of the drug. Previous exposure to AGS testing, which has been shown to reduce thresholds for electroshock and chemical convulsants in the AGS-prone rat strain (Consroe et al.,

1980), was not found to have similar effects in a normally resistant rat strain. This suggests that the repeated after-discharge of audiogenic convulsions may be necessary for the later elaboration of generalized susceptibility to various convulsogenics. While the present study did not demonstrate a significant effect of neonatal FZP on the occurrence of audiogenic motor seizures, it would be worthwhile exploring for such an effect in the genetic AGS susceptible strain.

While the underlying mechanisms involved in the FZP-induced resistance to PTZ-induced seizures are not known at present, a relatively long-term alteration of CNS function seems to be implicated, although peripheral effects, such as alterations of drug metabolism and hormone function cannot be discounted.

Alterations of hepatic microsomal enzyme activity, involved in drug metabolism, has been indirectly demonstrated in rats prenatally exposed to a BZ, as evidenced by significant differences in hexobarbital sleeping times (Buttar, 1980). Neonatal FZP exposure of rats in the present studies may have similarly altered drug metabolism, resulting in decreased convulsogenic effects of PTZ, and possibly PXN. Considering these reports it might be suggested that the long-term alterations related to

neonatal FZP are primarily motoric; regarding motor seizures, while still permitting the progressive development of focal seizures.

Hormonal disturbances occurring during a critical period of development can lead to immediate and/or delayed alterations of CNS structure and function influencing the development of convulsive disorders (Timiras & Hill, 1980). Since BZ's are known to cause hormonal disturbances (eg. sexual and reproductive behavior in adult rats, Buttar, 1980) one might speculate that similar hormonal alterations may have been involved in producing the present results.

It is also possible that neonatal BZ exposure alters brain or spinal cord function. Although there are few studies bearing directly on this question (eg. Kellog et al., 1980) there are reports that prenatal exposure of rats and human infants to BZ's produces a "floppy infant syndrome", characterized by hypotonia, poor suckling and spastic diplegia (Buttar, 1980; Speight, 1977).

The increased resistance to PTZ seizures in adulthood after neonatal FZP might also involve mechanisms in the CNS and/or spinal cord utilizing inhibitory GABA systems or BZ receptors themselves (Haefely, 1980; Guidotti, 1978). Pharmacological, physiological as well as developmental studies have demonstrated a close association between the

5

binding characteristics of BZ's and GABA, possibly involving a common receptor complex (for a review see Gallager, Mallorga, Thomas and Tallman, 1980; Killam & Suria, 1980). Furthermore, the epileptogenic aspects of PTZ, PXN and idiopathic epilepsy itself are increasingly suspected to involve both a reduction of synaptic inhibition and excessive increases in excitability due to inhibition of GABA-controlled paroxysmal activity (Killam & Suria, 1980; Myslobodsky & Valenstein, 1980; Woodbury, 1980). Inhibition of GABA catabolism and elevation of brain GABA levels using gamma-acetylenic GABA has been demonstrated to reduce or eliminate amygdala-kindled behavioral seizures and PXN-induced seizures (Myslobodsky & Valenstein, 1980). It has also been demonstrated that PTZ antagonizes GABA-mediated postsynaptic inhibition (Woodbury, 1980) and BZ binding (Antoniadis, Muller & Wollert, 1980). These phenomena may involve alteration of the GABA receptor-regulated chloride channel (Fujimoto & Okabayashi, 1981; Karobath, Drexler & Supavilai, 1981; Olsen & Leeb-Lundberg, 1981). Therefore, a prolonged increase in BZ receptor densities and/or enhanced GABA transmission may have resulted from the neonatal FZP exposure.

Suggestions for Future Research

The heightened resistance to PTZ and possibly PXN-induced seizures and slight attenuation of acoustic startle responses in neonatal FZP-exposed rats demonstrated here suggests that similar effects should be investigated in idiopathic seizure-prone rats. If AGS-prone strains of rats were examined, not only could the effects of early acoustic stimulation be more effectively evaluated, but long term effects of AGS susceptibility, in combination with neonatal BZ exposure could also be tested using chemical convulsants, as was done in the present study. It would also be useful to employ electrical brain stimulation-induced kindling, which would provide electrographic evidence of brain seizure activity which may establish whether or not neonatal FZP exposure provides increased protection against increased neuronal excitability or only blocks the behavioral manifestations of seizures. Another appropriate question is whether or not other known anticonvulsants other than the benzodiazepines (for example Diphenylhydantoin) have similar effects when administered in the immature rat.

Finally, it would seem worthwhile to investigate the neurochemical consequences of neonatal exposure to BZ's, particularly the effect of such exposure on the brain BZ /GABA receptor complex. In this regard it would seem

essential to compare pre- and postnatal exposure procedures and varying postnatal periods in search of a 'critical period' for inducing long term receptor changes. Conversely, the effects of similar regimens of exposure to chemical convulsants on brain BZ receptors merits investigation.

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Appendix

Table A

Effects of neonatal flurazepam (FZP) exposure upon audiogenic seizure (AGS) susceptibility and intensity in adult and young rats. Sprague-Dawley rats injected with 5 mg/kg FZP or saline (sc) on Days 1 to 15 were examined for AGS during 110 sec exposure to intense acoustic stimulation generated by a 109 dB electric bell mounted within a sound attenuating chamber. In a similar manner, neonatal Wistar rats received either saline or 5 mg/kg FZP on Days 1, 3, 5, 7 and 9, followed by AGS testing on Days 16, 18, 20, 22 and 24. Behavioral seizures were rated using a scale adapted from Jobe and associates (1973a) and described in Table 1 of the present report.

The results suggested that adult Sprague-Dawley and young Wistar rats exposed to FZP in infancy evidenced greater susceptibility to AGS.

Group	n	Age at Testing	No. Animals Susceptible	Maximum Intensity	n*
Sprague-Dawley					
Control	9	75 days	1	2	1
FZP	20	75 days	8	2 4	5 3
Wistar					
Control	10	16-24 days	2	1 4	1 1
FZP	10	16-24 days	4	1	4

Intensity 2 - wild running, 4 - tonic/clonic convulsions

* number of animals expressing seizure intensity specified.

Table B.

ASR ratings for neonatal FZP-exposed rats tested for AGS during the 7 trial procedure.

Group	Age at Testing	AGS Test Trials							
		Ss	1	2	3	4	5	6	7
Control	13 - 31	1	1	1	1	1	1	1	2
		2	1	1	1	1	1	1	1
		3	1	1	1	1	1	1	1
		4	1	1	1	1	1	1	1
		5	1	1	1	1	1	1	1
		6	1	1	1	1	1	1	1
		7	0	1	1	1	1	1	1
		8	1	1	1	1	1	1	1
		9	0	1	1	3	2	3	2
		10	1	1	1	1	1	1	1
		11	1	1	1	1	1	1	1
		12	1	1	1	1	1	3	3
FZP	13 - 31	1	0	1	1	1	2	2	2
		2	0	1	1	1	1	1	1
		3	1	1	1	1	1	1	1
		4	1	1	1	1	1	1	1
		5	0	1	1	1	1	1	1
		6	1	1	1	1	1	1	1
		7	0	1	1	1	1	1	1
		8	1	1	1	1	1	1	1
		9	1	1	1	1	1	1	1
		10	0	1	1	1	1	1	1
		11	0	1	1	1	1	1	1
		12	1	1	1	1	1	1	1

ASR 1 - startle response, 2 - single episode wild running
3 - 2 bouts wild running.

Animals tested for AGS on Days 26 - 44 and 39 - 57 received an ASR rating of 1 (startle response) on all 7 trials.

Table C

Seizure ratings for FZP-exposed rats and saline controls receiving repeated injections of PTZ

Group	Ss	PTZ Trials				
		1	2	3	4	5
Control						
AGS	1	3	4	3	4	4
	2	3	3	3	3	4
	3	3	4	4	3	1
	4	3	3	3	4	4
	5	3	3	3	1	3
	6	4	3	1	3	3
NoAGS	1	2	3	3	4	1
	2	3	4	3	3	3
	3	2	2	4	4	3
	4	3	4	4	4	4
	5	3	3	4	4	4
	6	3	3	1	3	3
FZP						
AGS	1	3	1	2	3	4
	2	3	3	3	4	3
	3	3	3	3	3	4
	4	3	2	3	1	3
	5	1	1	3	3	4
	6	3	2	3	4	4
NoAGS	1	3	3	2	3	4
	2	3	3	3	4	4
	3	3	3	2	3	2
	4	3	3	3	1	4
	5	3	2	1	3	2
	6	3	3	3	3	3

3 - myoclonic seizure, 4 - tonic/clonic convulsion

Table D

Seizure ratings for FZP-exposed and control rats
during PXN kindling

Group	Ss	PXN Trials									
		1	2	3	4	5	6	7	8	9	10
Control											
AGS Female	1	3	4	1	4	1	D	-	-	-	-
	2	D	-	-	-	-	-	-	-	-	-
	3	4	1	4	4	3	4	D	-	-	-
	4	1	4	4	4	D	-	-	-	-	-
	5	1	4	4	4	4	D	-	-	-	-
	6	4	4	4	D	-	-	-	-	-	-
Male	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	2	2	2	2	2	2	2
	3	1	1	1	1	1	1	1	1	1	1
	4	1	1	1	1	1	1	2	2	2	2
	5	1	1	2	3	3	2	3	3	3	3
	6	1	1	1	1	1	1	2	3	3	2
NoAGS Female	1	4	4	D	-	-	-	-	-	-	-
	2	4	4	4	4	D	-	-	-	-	-
	3	4	1	4	D	-	-	-	-	-	-
	4	4	4	4	D	-	-	-	-	-	-
	5	4	1	4	4	4	4	D	-	-	-
	6	1	1	4	4	D	-	-	-	-	-
Male	1	1	1	1	1	1	2	2	2	2	3
	2	1	1	1	1	2	2	2	2	1	3
	3	1	1	1	1	2	2	2	2	3	1
	4	1	1	1	1	2	2	2	2	2	3
	5	1	1	2	2	2	2	2	3	3	3

(continued)

Table D (continued)

FZP

AGS Female	1	4	4	4	4	D	-	-	-	-	-
	2	4	4	1	4	1	4	D	-	-	-
	3	4	4	1	3	4	D	-	-	-	-
	4	4	4	4	4	3	4	4	D	-	-
	5	4	4	D	-	-	-	-	-	-	-
	6	4	4	3	4	4	D	-	-	-	-
Male	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	2	2	2	1	1
	3	1	1	1	1	1	2	2	2	1	2
	4	1	1	1	1	1	1	1	1	1	1
	5	1	1	1	2	2	2	2	2	1	2
	6	1	1	1	1	1	1	1	1	1	1
NoAGS Female	1	4	4	1	D	-	-	-	-	-	-
	2	D	-	-	-	-	-	-	-	-	-
	3	4	4	4	3	4	4	D	-	-	-
	4	4	4	4	4	D	-	-	-	-	-
	5	1	4	4	4	D	-	-	-	-	-
	6	4	1	4	4	1	4	4	D	-	-
Male	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	1	1	1
	3	1	1	1	1	1	1	1	1	1	1
	4	1	1	1	1	1	1	1	2	2	2
	5	1	1	2	2	3	3	3	3	3	3
	6	1	1	1	1	1	1	1	1	1	1

D - death due to seizure (tonic/clonic)
See Table 6 of the text for rating scale

Table E

ANOVA summary of between group differences
in body weights at 10 days of age

Source	SS	df	MS	F	p<
Drug	202.71	1	202.71	26.12	.001
Sex	61.24	1	61.24	7.89	.005
D X S	3.39	1	3.39	.44	.510
ERROR	1644.99	212	7.75		

Levene's Test for equality of variances, $F(3,212) = .53$, $p < .6592$

Table F

ANOVA summary of between group differences
in body weights of rats at 13, 26 and 39 days

Source	SS	df	MS	F	p<
Age	.16937 E.06	2	84686.00	1540.89	.001
Drug	.62.55	1	62.55	1.14	.290
Sex	2396.00	1	2396.00	43.60	.001
A X D	115.80	2	57.90	1.05	.355
A X S	3965.00	2	1982.50	36.07	.001
D X S	147.13	1	147.13	2.68	.107
A X D X S	13.53	2	6.77	.12	.680
ERROR	3297.50	60	54.96		

Table G

Hotelling's T square summary of between group differences in the development of AGS as determined by the number of trials to the first occurrence of successive ASR ratings

Multivariate
Statistics

Mahalanobis D square	1.2595
Hotelling's T square	7.5569
F(3,20) value	2.2900
p value	.1090

Differences on Single Variables

(1) Startle Responses -	T(22) = 1.77, $p < .09$
(2) Single Wild Run -	T(22) = .76, $p < .45$
(3) 2 Bouts Wild Run -	T(22) = 1.43, $p < .17$

Table H

MANOVA summary of between group differences in
susceptibility to PTZ-induced seizures

Multivariate Test of Equality of Mean Vectors:

Neonatal Drug Treatment -	$F(2,19) = 4.512, p < .025$
AGS Testing -	$F(2,19) = .209, p < .813$
NDT X AGST -	$F(2,19) = 1.569, p < .234$

Significance Tests on Individual Variables

Neonatal Drug Treatment -

Myoclonic Seizure-	$F(1,20) = .122, p < .731$
Tonic/Clonic Seizure-	$F(1,20) = 9.009, p < .007$

AGS Testing -

Myoclonic Seizure-	$F(1,20) = .122, p < .736$
Tonic/Clonic Seizure-	$F(1,20) = .360, p < .555$

NeoDrug X AGS Testing -

Myoclonic Seizure-	$F(1,20) = 3.049, p < .096$
Tonic/Clonic Seizure-	$F(1,20) = .090, p < .767$

Table I

MANOVA summary of between group differences in susceptibility to PXN-induced seizures in female rats

Multivariate Test of Equality of Mean Vectors:

Neonatal Drug Treatment	-	$F(2,19) = 1.622, p < .224$
AGS Testing	-	$F(2,19) = .434, p < .654$
NDT X AGST	-	$F(2,19) = .496, p < .617$

Significance Tests on Individual Variables:

Neonatal Drug Treatments -

Tonic/Clonic Seizure	-	$F(1,20) = 2.353, p < .141$
Death	-	$F(1,20) = .534, p < .474$

AGS Testing -

Tonic/Clonic Seizure	-	$F(1,20) = .000, p < 1.0$
Death	-	$F(1,20) = .882, p < .359$

NeoDrug X AGS Testing -

Tonic/Clonic Seizure	-	$F(1,20) = .588, p < .452$
Death	-	$F(1,20) = .272, p < .608$

Table J

MANOVA summary of between group differences in susceptibility to PXN-induced seizures in male rats

Multivariate Test of Equality of Mean Vectors:

Neonatal Drug Treatment -	$F(2,18) =$	2.081,,	$p < .154$
AGS Testing -	$F(2,18) =$	.176,	$p < .840$
NDT X AGST -	$F(2,18) =$	2.827,	$p < .086$

Significance Tests on Individual Variables:

Neonatal Drug Treatment -

Myoclonic Jerks -	$F(1,19) =$	4.377,	$p < .050$
Myoclonic Seizures -	$F(1,19) =$	2.762,	$p < .113$

AGS Testing -

Myoclonic Jerks -	$F(1,19) =$	.240,	$p < .630$
Myoclonic Seizures -	$F(1,19) =$	.370,	$p < .350$

NeoDrug X AGS Testing -

Myoclonic Jerks -	$F(1,19) =$	1.861,	$p < .189$
Myoclonic Seizures -	$F(1,19) =$	.093,	$p < .763$

END

0 6 0 6.8 3

FIN