The Behavioural Effects of Benzodiazepines Following Metrazol-Induced Seizures.*

by Shirley Munk (C)

*This research was supported by Operating Grant #A0321 from the Natural Sciences and Engineering Council of Canada to K. Paul Satinder.

ISBN 0-315-34824-0

Abstract

Previous research indicates that seizures cause transient and in some cases, long lasting increases in the density of benzodiazepine receptors in the brain. The present research sought to discover the behavioural effects of diazepam (Valium), one of the benzodiazepines, on rats in a conflict paradigm following Metrazol induced seizures. A total of 82 SHS rats (both sexes) were used in this 4(diazepam doses) X 2 (seizure condition) X 2(sexes) factorial design. The conflict involved the availability of food for 24 hour food deprived rats in a brightly lit open field . Since diazepam is known to have an anticonflict effect, it was hypothesized that animals experiencing a seizure would demonstrate a greater anticonflict response. Results showed significant main effects for diazepam and seizure factors in food eaten, approaches to the food and in the ratio between food eaten and approaches to the food. However, the seizure condition animals showed less anticonflict behaviour which is contrary to what was predicted. Significant drug by seizure interactions were expected and confirmed. The possible drug interactions at the level of the brain receptors is discussed, as well as, the behaviour resulting from combining central nervous system(CNS) stimulants with CNS depressants and anxiolytics.

ACKNOWLEDGEMENTS

I wish to acknowledge the contributions of Dr. Paul Satinder, my advisor, Dr. Steve Goldstein and Dr. John Jamieson to the completion of the thesis. Their advice and suggestions were greatly appreciated.

Laraine Pringle and David Gregory were helpful assistants whose contributions were very much appreciated.

Completion of this project would have not been possible without the support of many friends. Lorraine and Ian Cull and Michael and Linda Nolan were invaluable friends and I thank them for being there. A special thanks goes to Michael Nolan, my friend and co-worker. I would also like to thank Dr. Nolan for helping obtain the pharmaceuticals needed for my work. As always, my family was a great source of support and I thank them for continuing to have faith in my abilities.

This thesis is dedicated to Michael.

TABLE OF CONTENTS

	Page
ABSTRACT.....	ii
ACKNOWLEDGEMENTS...	iii
TABLE OF CONTENTS.....	iv
LIST OF TABLES AND FIGURES...	
INTRODUCTION.....	
PHARMACOKINETICS AND PHARMACODYNAMICS OF BENZODIAZEPINES.....	
BENZODIAZEPINE RECEPTORS...	
ONTOGENY OF THE RECEPTORS.....	
BEHAVIOURAL EFFECTS OF BENZODIAZEPINES...	
RATIONALE FOR PROPOSED RESEARCH.....	15
METHOD...	17
RESULTS.....	22
DISCUSSION...	32
REFERENCES...	45

LIST OF TABLES AND FIGURES

Table — Page

Four operant paradigms, neurotransmitter correlates and antagonistic drugs.....

F values for Main Effects by Themselves and with Covariates of Weight, Age and Weight and Age Together for Amount of Food Consumed..... 24

Figures

The Diazepam molecule.....

The benzodiazepine-GABA receptor chloride ionophore complex.....

Means and Standard Deviations of Food According to Seizure and Drug Dosage.....

Means and Standard Deviations of Approaches According to Seizure and Drug Dosage..... 26

Means and Standard Deviations of Activity According to Seizure and Drug Dosage..... 28

Means and Standard Deviations of the Ratio of Food Consumed Per Approach According to Seizure and Drug Dosage..... 29

Arousal Hypothesis of Hyponeophagia..... 41

Introduction

With the discovery of opiate receptors by Snyder and Pert in 1973, investigations into neurophysiological mechanisms of behaviour have delved into the development and manipulation of these and other receptors. Benzodiazepines, which are known for their anxiolytic, anticonvulsant and sedative effects, also have specific receptors in the brain (Squires & Braestrup,1977; Mohler & Okada,1977). While there have been successful attempts at modulating these receptors, either by increasing or decreasing the number of them in rat brain specimens, the resultant behavioural effects have not been widely studied. Enhancing the knowledge in this area may help to explain individual variability in response to these drugs, and perhaps give insight into the effects of combining various therapies such as electroconvulsive shock and neuroleptics.

Pharmacokinetics and Pharmacodynamics of Benzodiazepines

Classed as minor tranquilizers, benzodiazepines first appeared on the market as chlordiazepoxide (Librium®) in 1960. Diazepam (Valium®) was developed in 1962 and has become the most widely known and prescribed benzodiazepine (Hollister,1983; Ramsey,1982).

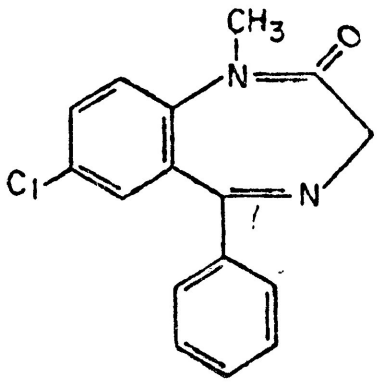

Fig. 1. The Diazepam molecule

Diazepam, the most commonly used benzodiazepine, is administered orally predominantly and it's onset of action occurs in 30 to 60 minutes in humans. Intramuscular or subcutaneous injection is used as a preoperative anxiolytic and sedative but this drug is better absorbed through the gastric mucosa. Taken orally, plasma concentrations peak in 2 to 4 hours, and metabolism takes place in the liver. Excretion is

predominantly accomplished in the urine (Govoni and Hayes,1978). Intravenous injection is frequently used in the treatment of status epilepticus because of it's fast and long duration of action. Clonazepam is useful in chronic treatment in certain types of seizures (Rall & Schleifer in Gilman et al.,1980) The metabolism of benzodiazepines is unique in that it occurs in two phases, accounting for it's long duration of action. The initial distribution of diazepam, for example, takes about 1 hour to occur, alleviating the symptoms rather quickly. The active metabolite of diazepam, norazepam, can take up to 1.5 days to be totally eliminated from the body (Baldessarini in Gilman et al,1980; Rickels,1982). Careful maintenance of blood levels must be kept in order to avoid toxicity and establish a minimum effective dose for each individual's needs.

With the discovery of these substances came the interest in exactly how this unique drug works in the brain and the central nervous system. Haefely et al.(1975), first proposed that the neurotransmitter involved with this drug's action was Gamma aminobutyric acid (GABA), the primary inhibitory transmitter in the brain and other parts of the nervous system. An amino acid with neurotransmitter properties, GABA mediates the inhibitory actions of neurons in the cortex,midbrain and cerebellum and has some action within the spinal cord. The areas in which GABA is found in high concentrations include the cerebellum, the olfactory bulb, the cuneate nucleus,

hippocampus, the lateral septal nucleus and between the vestibular nucleus and the trochlear motoneurons (Bloom in Gilman, Goodman & Gilman,1980) Considering the heavy GABA involvement in the limbic system and the anxiolytic properties of benzodiazepines there is likely to be a relationship between the two substances. A model of this relationship is represented by the benzodiazepine-GABA receptor chloride ionophore complex as proposed by Paul and Skolnick(1982) and shown below.

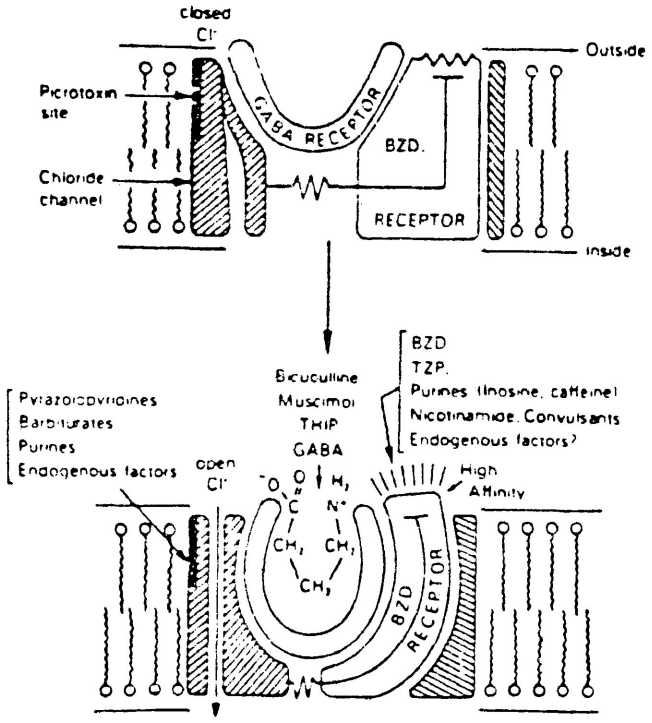

Fig. 2. The benzodiazepine-GABA receptor chloride ionophore complex.(From Paul & Skolnick, 1982,p. 38.)

GABA, which originates from glutamic acid is stored in the synaptic vesicles of GABAergic neurons. Once released, it can

be taken up by the presynaptic membrane from which it came or it can become attached to GABA receptors on the postsynaptic membrane. Chloride channels open when GABA receptors are stimulated so that two events may follow. If there is a high concentration of Cl^- extracellularly, hyperpolarization of the membrane will occur as the negative chloride ions rush into the cell. Conversely, if there is a high concentration of Cl^- intracellularly, the membrane will depolarize. Instances of depolarization lead to presynaptic inhibition such as in the case of axo-axonic synapses. Similarly hyperpolarization precedes postsynaptic inhibition. Because of the nature of GABA, the net effect is inhibitory in the nervous system (Haefely,1983). Benzodiazepines, and specifically the recently discovered benzodiazepine receptor (Squires and Braestrup,1977; Mohler and Okada,1977), are thought to enhance the action of GABA by increasing the membrane's permeability to chloride ions (Gallager, Mallorga, Thomas and Tallman,1980). Research has been done to investigate the effects of benzodiazepines at many levels of the neuroaxis where inhibitory action is found. Schmidt et al.(1967) found that diazepam facilitated presynaptic inhibition in the cat spinal cord. Studies investigating the enhancing effects of benzodiazepines on postsynaptic inhibition were done on the cuneate nucleus by Polc and Haefely (1976), and diazepam has been found to increase the frequency of ion channel openings in cultured mouse spinal cord (MacDonald and Barker,1978). The GABAergic synapses are found

in the hippocampus and amygdala; benzodiazepines again modulating GABA systems. Lee et al. (1979) found an increase in GABA action in hippocampal slices when they are exposed to diazepam. At the higher cortical levels, Raabe and Gumnit (1977) studied the effect of diazepam on postsynaptic inhibition in the cat motor cortex, finding the usual suppression of depolarization.

The anticonvulsant effects of benzodiazepines are easily explained in terms of the mechanism of action of this substance. By facilitating the release of GABA, inhibition of firing of the neurons can occur, preventing massive, uncontrolled firing. This is most readily demonstrated with convulsant drugs which block GABA transmission, such as in the case of bicuculline, picrotoxin or pentylenetetrazol. Benzodiazepines do not work on an epileptic focus per se, but rather prevent the spreading depolarization that occurs (Rall & Schleifer in Gilman et al., 1980).

Benzodiazepine Receptors

Using tagging measurement techniques, in which radioactive $^{3}[H]$ is bound to receptor sites, much has been learned about the benzodiazepine receptor in the last decade. This receptor is protein in composition, has a molecular weight between 50,000 and 60,000 daltons and is found on the outer membrane of various neurons (Braestrup and Nielsen, 1981). An explanation of a technique using $^{3}[H]$ diazepam to identify the

location of receptors, is provided by Mohler and Richards (1983). One of the first to discover these receptors, Squires and Braestrup (1977), found binding to be 3 to 4 times higher in the frontal and occipital cortices than in the pons medulla. Intermediate binding levels were found in the hippocampus. Similar work was done by Mohler and Okada (1977). They quantified the density of ^{3}[H] diazepam specific binding sites at different parts of the brain, finding the highest density in the cerebral cortex (305± 10 fmole/milligram of protein). Values (same unit as above) of (290±22) in the hypothalamus, (270±21) in the cerebellum, (269±15) in the midbrain, (252±13) in the hippocampus, (180±13) in the striatum, (162±22) in the medulla-pons and (90±22) in the spinal cord show the relative distribution of the receptors. Although there does not appear to be regional variations in the binding affinity of benzodiazepines, there are differences in the affinities with different benzodiazepines (Mohler and Richards, 1983). The more potent the benzodiazepines are stronger inhibitors of ^{3}[H] diazepam binding than weaker ones, the stronger ones also having more effect physiologically and behaviourally, for example, clonazepam, lorazepam, flunitrazepam and diazepam (Mohler and Okada, 1977).

Ontogeny of the Receptors

Binding sites for benzodiazepines have been found in rats at 14 days of gestation at about 5% the adult binding level, increasing to between 20-26% at birth (Gallager et al., 1980; Braestrup and Nielsen, 1978). By 21 days of age, adult levels have been acheived

and not surprisingly, the development of benzodiazepine receptor sites coincides with the development of GABA receptors (Coyle and Enna, 1976).

File and Tucker (1984) describe two distinct phases of benzodiazepine receptor development and attempt to show the possibility of two types of receptors: one which modulates the anxiolytic effects and one which has different properties (Lippa et al.,1981). Before Day 14 the immature system of the rat causes phenytoin, (an anticonvulsant which is normally inhibitory), to be excitatory and benzodiazepines at this stage are likely to produce convulsions (Barr & Lithgow,1983). It is during this time of plasticity that File and Tucker tried to produce lasting effects in the behaviour of animals treated with CGS 8216, a substance that displaces benzodiazepines from binding sites but has opposite effects behaviourally. They found that animals treated with this drug showed an increase in social interaction and increased susceptibility to convulsions thus demonstrating that anxiolytic and anticonvulsant actions may be governed by different mechanisms. Research is needed to discover more about this possibility of differential development of the benzodiazepine receptor.

Attempts have been made at modulating the benzodiazepine receptor in the developing animal as well as in the adult animal. Exposure in utero to diphenylhydantoin (an anticonvulsant) between Day 14 and Day 20 resulted in benzodiazepine binding decreases at Day 14 and Day 21 postnatally but these changes were not permanent as levels returned to control levels by Day 28 (Gallager

and Mallorga, 1980). Gallager (1982) reported that shock administered to pregnant rats during the stage of proliferation of binding sites in the pups, decreased binding sites in the offspring postnatally but no changes were noted in receptor affinity. In the same paper, Gallager described the effects of a benzodiazepine agonist (clonazepam), and an antagonist (RO15-1788), on binding sites. She found that prenatally administered clonazepam failed to produce any significant effect on the site density or seizure threshold and that RO15-1788 decreased the density of the sites and also decreased the seizure threshold in the offspring.

In adult animals alteration in benzodiazepine receptor density has also been reported. Long term treatment with benzodiazepines and their agonists has shown a small but significant decrease in the number of binding sites but in some cases a tolerance to the effects has been differential. Braestrup, Nielsen and Squires (1[illegible] cited in Braestrup & Nielsen, 1981), state that tolerance seems to develop to the sedative and anticonvulsant effects but not to the anxiolytic effects. In another study in which mice were treated with either diazepam (up to 100mg/kg p.o.) or lorazepam (up to 60 mg/kg p.o.), Braestrup and Nielsen (1981), failed to show an increase in the benzodiazepine sites. They did, however, find a functional reduction in the interaction between benzodiazepine receptors and GABA receptors.

Diphenylhydantoin has been shown to produce a dose dependent increase in the total number of benzodiazepine binding sites and also to improve the action of diazepam in controlling

spontaneous firing in the dorsal raphe cells in the brain (Gallager, Mallorga and Tallman,cited in Mennini and Garattini,1982). As Mennini and Garattini point out, in the three studies they cite, there is a consistent 10-25% increase in benzodiazepine sites after chemical manipulation, and this would seem to imply that the number of binding sites may be important in terms of the actions both physiologically and behaviourally. The effects of seizures induced electrically or chemically have shown increases in site density but not in the affinity of diazepam for the receptors. Paul and Skolnick (1978) used convulsive shock (150V,1 sec., A.C.), subconvulsive shock (70V 0.4 sec., A.C.), sham-shock, pentylenetetrazol injection (45mg/kg in 0.9% saline) and saline injection alone on adult male rats. The amount of ^{3}[H] diazepam bound to cerebral cortical membranes increased after tonic/clonic seizures induced by electric shock (21.2% at 15 mins. and 21.4% at 30 mins.) and the same increases occurred with pentylenetetrazol. By 60 minutes the levels returned to their preseizure levels. Subconvulsive shock did not cause a change in the number of sites. Increases in receptor binding that are longer lasting have been found with studies using kindling rather than generalized seizures (McNamara, Peper and Patrone,1980). The results showed that a graded response occurs in which the greater number of kindled seizures produced a systematic increase in receptor sites Repeated hypoxia (a potential confounding factor) and repeated electrical current without

seizure did not cause an increase. Binding was increased by 35% with 16.3 stimulations in both the right and left hippocampi in comparison to controls. Two groups of animals received repeated electroconvulsive shock (nonkindled) where the seizures are more intense. One group received an average of 17 seizures and showed a 17% increase in receptor binding, while the second group had only 7 seizures and showed an insignificant rise of only 8%. These results show that seizure activity does cause alterations in the number of binding sites and could therefore alter the effects that further exposure to benzodiazepines (endogenous or exogenous) might have on the physical and behavioural response of the organism.

Behavioural Effects of Benzodiazepines

The pharmacodynamics of benzodiazepines are diverse, producing physiological, as well as, behavioural effects. Acting on motor neurons in the spinal cord, benzodiazepines may act as muscle relaxants promoting presynaptic inhibition (Hollister,1982) and these are also known to have sedative as well as hypnotic properties. The most important uses for these drugs are, however, for the attenuation of anxiousness and for it's anticonvulsant activity. In these cases, the facilitation of GABA release in the hippocampus and related limbic structures, is likely the mechanism by which these effects occur although there is evidence to suggest that there may be two different

kinds of benzodiazepine receptors, each having different mechanisms of action (Klepner, et al., cited in File,1981).

The anxiolytic effects of benzodiazepines have been studied using both animal and human models. In clinical use, benzodiazepines have been found to relieve anxiety and tension. Lader (1981) states that patients taking these drugs experience less emotion of all types, and not only anxiety, so it would seem that benzodiazepines tend to "level out" emotions with a very unspecific action. A compilaton by Linnoila (1983) of the research done on the psychomotor effects of diazepam show that it impairs performance on tasks such as tracking and divided attention, as well as, increasing the critical flicker frequency. Acquisition of information can be impaired but retrieval may be improved with diazepam.

Anxiety in animals and the effects of benzodiazepines have been studied in detail. Haefely (1983) describes the effects as that of an anticonflict or antipunishment effect; behaviour that is surpressed is released from suppression. A problem in the past was that of trying to induce "anxiety" in animals and expect that the behaviour will be similar to the behaviour expressed in humans. But models have been found that do demonstrate the anxiolytic effects of benzodiazepines. Sandra File (1981) developed the social interaction test of anxiety with rats in which the amount of interaction between two

male rats can be varied depending on environmental test conditions. These variable conditions include amount of illumination in the test area and familiarity of the subjects with the test chamber. This test is useful because it distinguishes between the sedation and anxiolytic effects of benzodiazepines. File and Vellucci (1979) measured corticosterone levels (an indicator of how much stress the animal is experiencing) in rats under two different illumination conditions (high and low) and also in familiar and unfamiliar circumstances and found that the highest levels of corticosterone were found in animals who were in the high light/unfamiliar condition (84.6 $\pm$5.75ug/100ml) compared with the low light/familiar condition (41.8 $\pm$4.25ug/100ml). When given chlordiazepoxide (5mg/kg for each of the five days) the rats in the high stress situation showed lowered corticosterone levels, as well as, increased social interaction scores (403 secs. as compared to 227 secs. for untreated animals).

Other models that generate the effects of benzodiazepines have been outlined by Larry Stein (1982). Displaying the four possible circumstances in operant learning, Table 1. shows the drug group that would antagonize the effect. The neurotransmitter linked to these behaviour patterns is also depicted.

Table 1. Four operant paradigms, neurotransmitter correlates and antagonistic drugs (From Stein, 1982, p. 384)

Consequence	Presentation	Omission
Favorable	Positive Reinforcement	Nonreward
	(facilitates behavior)	(inhibits behavior)
	Phenothiazines	Benzodiazepines
	Catecholamines	Serotonin,Acetylcholine
Unfavorable	Punishment	Negative Reinforcement
	(inhibits behavior)	(facilitates behavior)
	Benzodiazepines	Phenothiazines
	Serotonin,Acetylcholine	Catecholamines

The benzodiazepines are useful to release behaviour suppressed by punishment or nonreward and work on a different neural system than the phenothiazines which can reverse positive or negative reinforcement effects. Stein et al. (1975) showed that benzodiazepines induced increases in the rate of punished responses in a rat conflict test where hungry rats perform a lever press response to obtain a food reward. Similar results were found by Vogel, Beer and Clody (1971) in an experiment where footshocks were delivered to rats for drinking water. The response rate during the punishment phase is also dose dependent, the higher doses showing the greatest effect. An interesting experiment by Britton and Britton (1981) utilized a brightly lit open field apparatus with a piece of food placed in

the center of the arena. The proposed conflict model comprised the rat's hunger vs it's tendency to respond with caution in a strange or new environment (ie., it will avoid the center and stay close to the perimeter). The dependent measure which showed the typical benzodiazepine effect was the amount of food eaten (g)/per number of approaches to the food in the center of the field. Leonard Cook (1982) has also shown this effect in humans using monetary rewards in a conflict pardigm.

Rationale for Proposed Research

While short term and long term increases in benzodiazepine receptors have been noted after seizures (Paul & Skolnick, 1978; McNamara, Peper and Patrone, 1980), the literature indicating the behavioural effects have not been found by this reseacher. The purpose of the present research was to study the behavioural response to an expected rise in benzodiazepine receptors after a seizure. A valid question arises as to whether behaviour is related to benzodiazepine receptors (ie., do animals with more receptors display behaviours different from animals with fewer receptors?). One approach to answering this question would be to examine genetic strain differences as was done by Robertson (1979) and Robertson, Martin & Candy (1978). In these studies it was shown that in rats and mice selectively bred for high or low emotionality or reactivity, the animals that showed more fearfulness had fewer receptor sites than the less fearful animals and particularly in the limbic areas of the brain. Gentsch, Lichtsteiner & Feer (1981) found that benzodiazepine binding was greater in the Roman High Avoidance rats than in the Low Avoidance, concurring with the theory that less emotionality means greater numbers of receptors for benzodiazepines in certain parts of the brain.

These results seem to indicate that there is a relationship between receptors and displayed behaviour.

Diazepam is used often in the treatment of recurring seizures, because of it's ability to block uncontrolled firing and generalized seizure activity. If indeed the number of receptors increase with a seizure, it is possible that the drug will have an increasingly potent effect and cause more pronounced behavioural effects. In a conflict paradigm similar to Brittons' (1981) open field measure, an animal having undergone a seizure and later injected with diazepam should presumably show a greater anticonflict response by spending more time in an aversive situation.

This study then, proposed to examine the effects of a seizure on the behavioural responses to benzodiazepines (Diazepam) in rats. One genetic line from the Lakehead University Animal Laboratory, namely Satinder's Heterogenous Stock (SHS) (Satinder, 1980) was used in this study. Since this line is a 4 way cross among 4 genetic lines, (Roman Low and High Avoidance and Maudsley Reactive and Nonreative rats), the distribution of benzodiazepine receptors in the brain would be intermediate based on the previously mentioned work by Robertson (1979), Robertson et. al. (1978) and Gentsch et al. (1981).

Method

Subjects

A total of 82 SHS animals (both sexes) were used. The first group of 48 animals were tested at approximately 125 days of age, while the second group of 34 rats were tested a month later at approximately 75 days of age. Age was considered a variable in the analysis of the results. All subjects were bred and reared in the Lakehead University Laboratory and given food and water ad libitum. Details of the methods of care and handling can be found in Satinder and Hill (1974). The laboratory was maintained on a 12 hour light/dark cycle with the lights coming on at 8 am. Temperature in the laboratory was maintained at approximately 22 C (± 1 C).

Experimental Design

The design was a 4(3 doses of diazepam and saline control dose) X 2(seizure vs no seizure) X 2(sex) X 2(Age Group 1 or 2). Dependent measures included food consumed, approaches to the food, the number of squares crossed in the open field, a ratio of the grams of food eaten per approach, and body weight on the day of testing.

Apparatus

The open field was a modified version of the apparatus in place in this laboratory. One quarter of this field was used, thus the dimensions were 45 X 45 cm with walls approximately 45 cm high. Over head light was provided by 4 90cm florescent lights providing a light intensity of 230 ftc. Viewing of the activity in the chamber was made possible by a sliding Plexiglas door. To attenuate extraneous noise which may startle the animals, a white noise stimulator (Model 1421, Layfayette Instrument Co.) was used. The sound intensity was 65 dB. Fixed with tape in the center of the field, was a petri dish containing preweighed wet food. The food was prepared by adding approximately 3 parts water to 1 part Rat Chow and allowing the water to completely saturate the food. It was mixed to the consistency of a thick paste.

Procedure

Three days prior to testing, littermate pairs were assigned to their experimental group (one to the seizure group, the other to the non seizure group) balancing the groups for weight and were housed individually. Two days before testing each group of animals (16 animals were tested per day) was given experience with the wet food to avoid a neophagic effect in the testing situation. The dry food was removed and replaced with a petri dish filled with the wet food. Water was always available

ad lib. The day before the actual testing, the remaining food was removed so that each animal was food deprived 24 hours before testing in the open field. Animals were weighed on the testing day.

Seizure induction procedure. Animals assigned to the seizure group were weighed and carried in their cage to the room set up to videotape the seizures. The dose of convulsant, Pentylenetetrazol, was prepared according to the weight of the animal (50mg/ml/kg.) and administered intraperitoneally (I.P). A glove was worn to hold the animal for all injections. The animal was immediately placed in a plexiglas chamber and the seizure activity recorded for a 5 minute period. Later, the video tapes of the seizures were analysed. Six measures were determined for each seizure. The onset and duration of the first myoclonic jerk and of the most severe part of a seizure (the animal straightens forelimbs and is rigid) were recorded and scored as actual values in seconds. The durations of the four phases were determined and then multiplied by a factor so that each animal received a score for each phase.Phase 1 (myoclonic jerks) was multiplied by a factor of 1; Phase 2 (jump-like jerking movements) was multiplied by a factor of 3: Phase 3 (severe tonic/clonic posture, lying on one side) was multiplied by a factor of 6 and Phase 4 (described above) was multiplied by a factor of 10. The reason for using these factors was that, based on previous pilot studies, the severity of each of these phases varied. Based on the ratings of three

raters of the video taped seizures of 75 rats, it was determined that Phase 2 was three times as severe as that of Phase 1, Phase 3 was 6 times as severe as Phase 1 and Phase 4 was 10 times more severe than Phase 1. To give more of a weighting to Phase 4 than Phase 1, allowed more of a comparison of severity of the seizure than simply using the latency of each phase in actual time.

After 5 minutes the animal was removed from the observation chamber and observed for 20 minutes in a recovery cage before the second injection and testing in the open field. Because the literature indicates that the increase in receptors may be a transient phenomenon, it was necessary to complete the behavioural testing soon after the seizure. Most animals had returned to normal activity following the 20 minutes period. Animals not receiving the convulsant were given a saline injection I.P.

Open field testing procedure At the end of the 20 minute recovery period, each animal received one of the four doses of diazepam (0mg/kg, .75mg/kg,1.5mg/kg,3.0mg/kg) again balancing for weight. These levels were chosen as they are similar to levels found in the literature (Britton and Britton,1981). The drug (1ml/kg) was injected intraperitoneally. The animals then were moved to a quiet semidark area for 30 minutes while the drug was given time to act. At the end of the 30 minute period

the animal was placed in the open field chamber for 15 minutes and the following behaviours were noted. 1) number of squares crossed; 2) number of approaches to the food dish in the middle of the chamber; 3) amount of food eaten; 5) urination and defecation. After the 15 minute period was over the animal was returned to its cage on the rack and the residue food was measured to determine how much food was eaten.

Results

Results were evaluated by analysis of variance to examine the following components of behaviour: a) amount of food consumed; b) approaches to the food; c) activity (no. of sections crossed); d) food consumed per approach; and e) body weight. These measures were analysed in relation to the four independent measures: seizure condition, dosage of diazepam, sex and age group. Sex and age group differences in the various phases of the seizure were also examined as was the relationship between the seizure activity and the animals' performance in the open field. Where the data were not homogeneous, the nonparametric Kruskall-Wallis test was used to examine differences. The predetermined significance level for all findings was considered to be $p < 0.01$.

Amount of Food Consumed

In the initial factorial analysis of variance of food consumed, there were no significant differences due to age so that groups were pooled for further analysis, ie. all 82 animals were combined (there were 4 missing values because of death after seizure). The means for the amount of food consumed according to seizure condition and drug dosage appear in Fig. 3. A summary of the analysis is shown in Table 2 and from this Table it is clear that there is no sex difference independent of

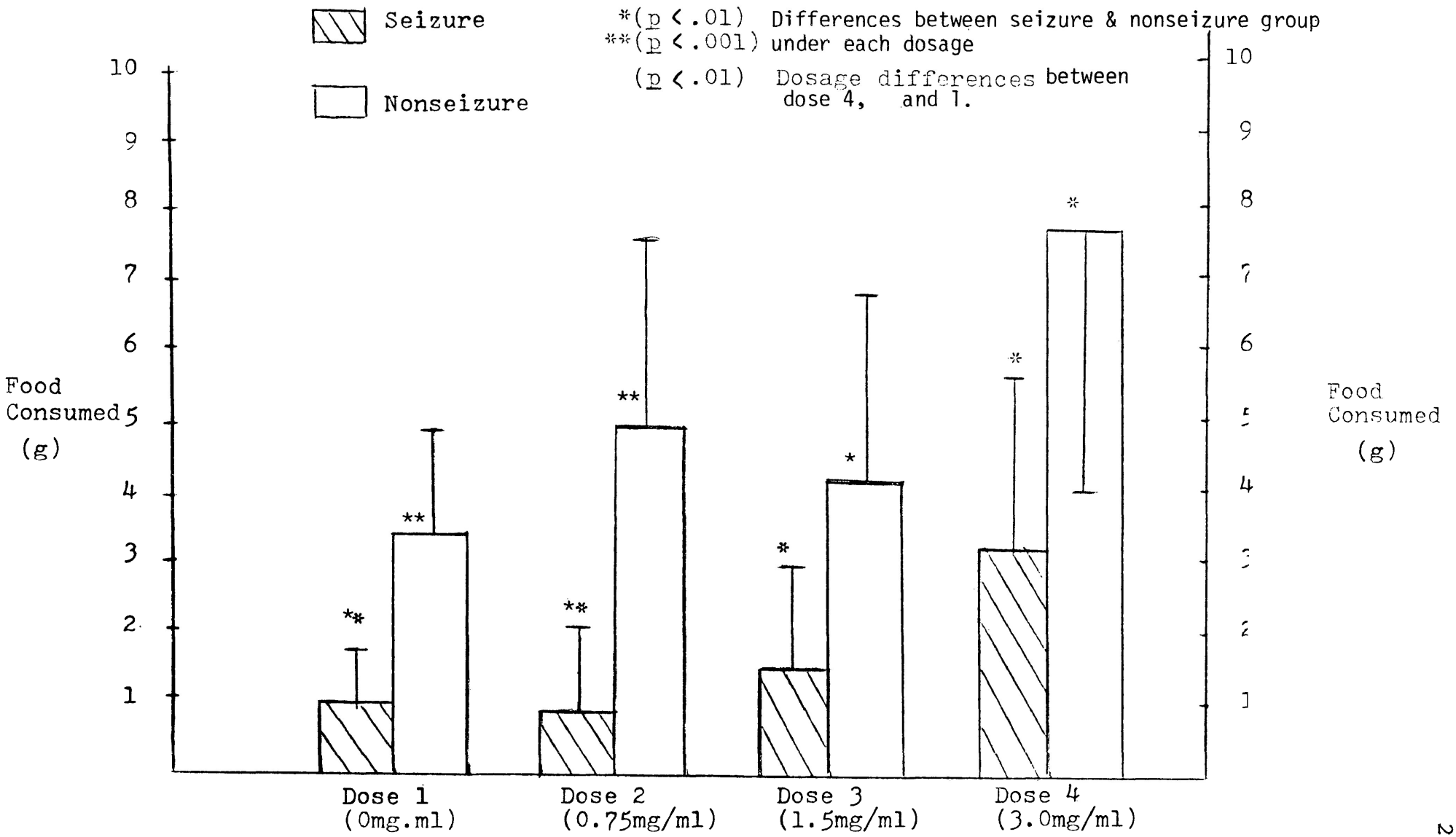

Fig. 3. Means and Standard Deviations of Food Consumed According to Seizure and Drug Dosage.

Table 2

F values for Main Effects by Themselves and with Covariates of Weight, Age and Weight and Age Together for Amount of Food Consumed.

Factor	*F* value	Covariates		
		Weight	Age	Weight & Age
Seizure	46.9** (1,62)	47.9** (1,61)	49.0** (1,61)	48.5** (1,60)
Dosage	9.0** (3,62)	9.1** (3,61)	9.2** (3,61)	9.2** (3,60)
Sex	9.4** (1,62)	5.4* (1,61)	8.8** (1,61)	0.2 (1,60)

* ($p < 0.05$)

** ($p < 0.01$)

body weight and age. Oneway analysis of variance for Dose 3 and 4 showed that the seizure condition animals ate significantly ($p< .01$) less food than animals not receiving a seizure. Nonparametric tests were used with analysis of Doses 1 & 2, and they also revealed that significantly ($p< .001$) less food was consumed by the seizure animals. There were no significant differences among any of the dosages in the seizure group in terms of food consumption. However, animals not receiving a seizure did vary. Kruskall-Wallis analysis of the differences between Dose 1 & 4 showed that animals who received Dose 1 ate less food than those in the Dose 4 group. All other combinations were nonsignificant.

Approaches to the Food

With the number of approaches, no significant sex or age differences were found. An interaction was found between seizure condition and drug dosage ($F(3,46)= 4.8$, $p<.01$) and this interaction also occurred when the sexes and ages are combined ($F(3,70)= 3.8$, $p<.01$). Fig. 4. shows the means for approaches according to seizure condition and drug dosage. Animals in the seizure group generally made fewer approaches to the food than the animals not receiving a seizure. Oneway analysis of variance showed that the seizured animals made significantly ($p<.01$) fewer approaches under Dose 1 and 2, but the seizure condition had little effect under Doses 3 and 4. A oneway

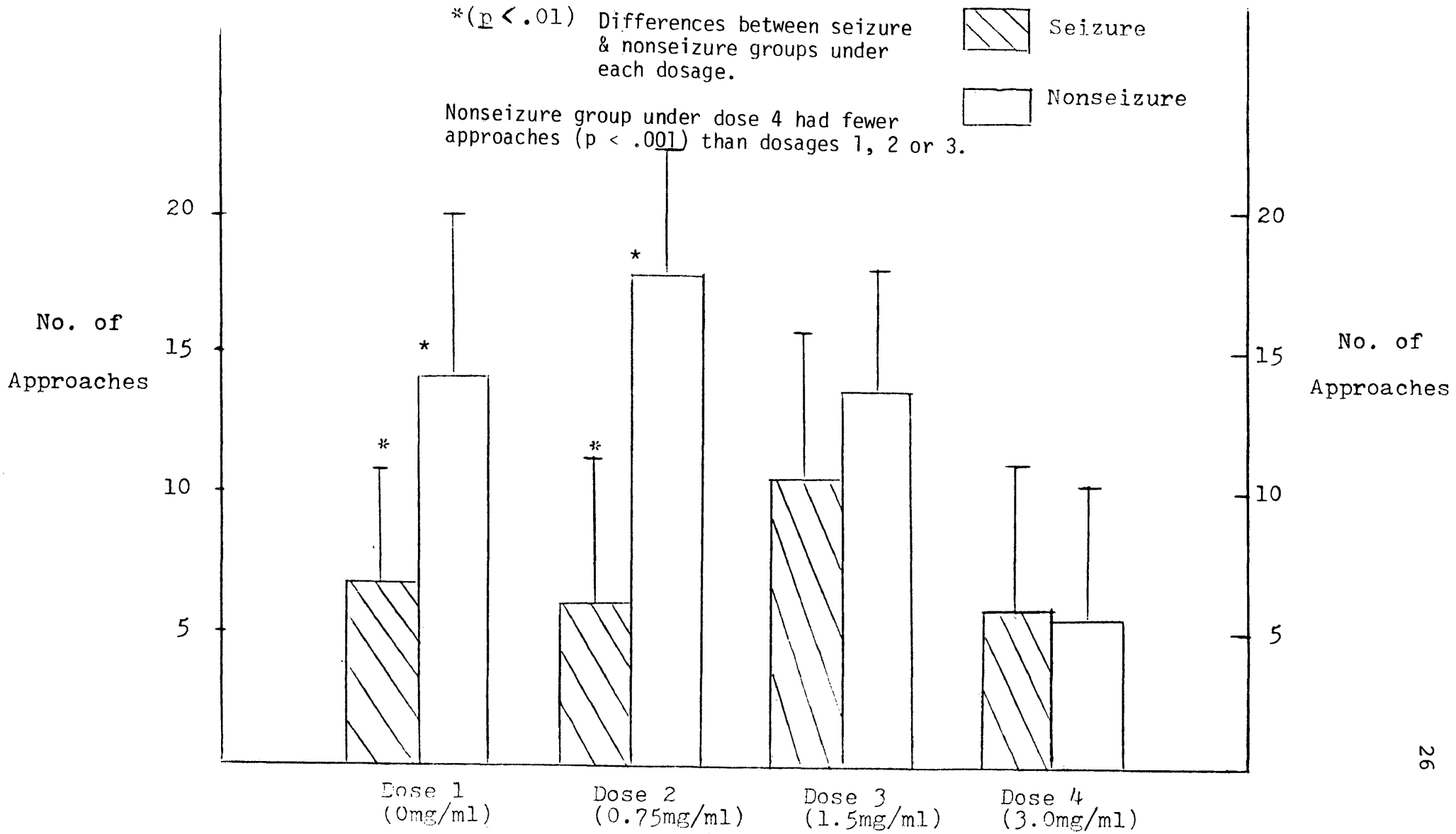

Fig. 4. Means and Standard Deviations of Approaches According to Seizure and Drug Dosage.

analysis of variance was also conducted for each of the two seizure conditions separately. There were no significant differences amongst 4 dosages in the seizure animals, but animals receiving Doses 1, 2 and 3 in the nonseizure group made significantly ($p<.001$) more approaches to the food when compared to animals under Dose 4.

Activity

The only significant main effect in activity score was that of drug dosage ($F(3,46)= 5.6$, $p<.01$). Figure 5. shows the means and standard deviations according to seizure condition and drug dosage. Animals receiving Dose 4 regardless of seizure condition showed less activity than the animals under the other three dosages.

Ratio of Grams of Food Eaten Per Approach

Referring back to original raw data, there was one male rat in Group 2 that had a ratio score of 10, which is an extreme score when considering a mean of 0.76 for all males in the study. Therefore the data was analysed excluding this case, which caused the mean for males to drop to .53. Fig. 6. shows the means and standard deviations according to seizure condition and drug dosage. Analysis of variance revealed significant seizure and dosage differences but no sex or age differences. A

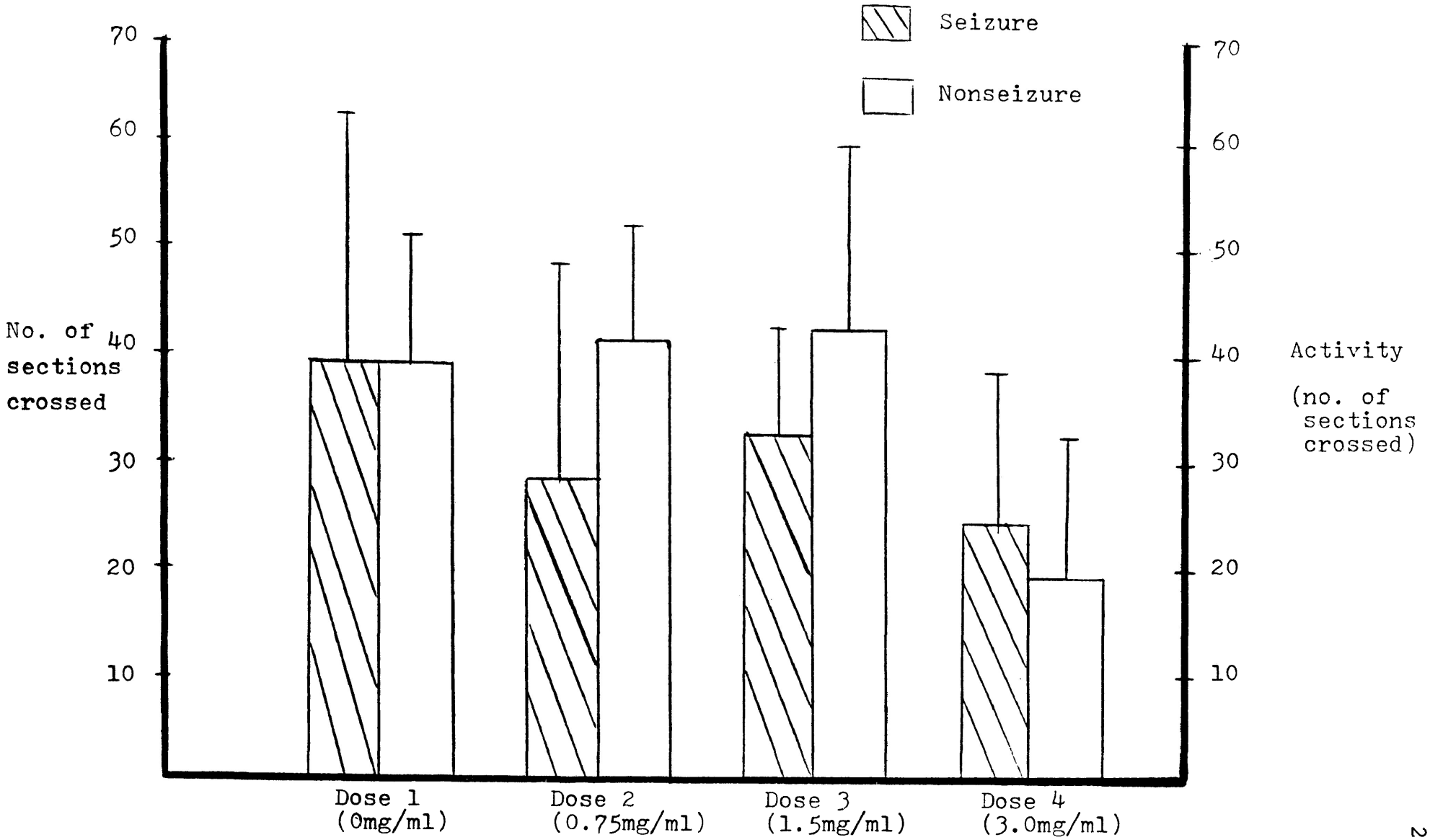

Fig. 5. Means and Standard Deviations of Activity According to Seizure and Drug Dosage.

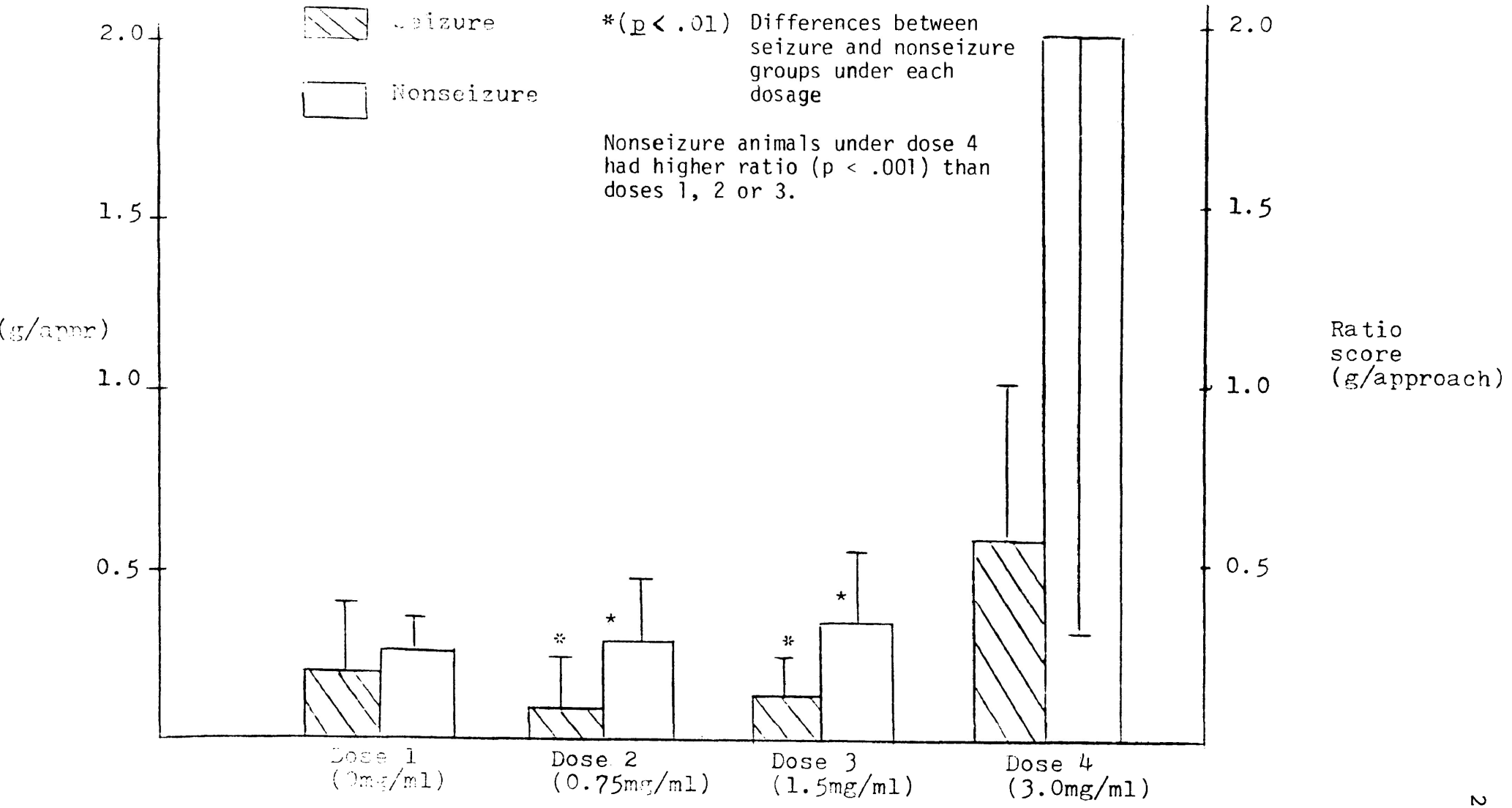

Fig.6. Means and Standard Deviations of the Ratio of Food Consumed Per Approach According to Seizure and Drug Dosage.

seizure condition X drug dosage interaction was also present $F(3,61)= 4.7$, $p<.01$). Generally animals receiving a seizure had lower ratio scores than nonseizure animals and those receiving Dose 4 of diazepam (in their respective seizure conditions) had a higher score than animals in any other of the three dosages. The animals receiving a seizure did not differ significantly from one dosage to another when a oneway analysis of variance or nonparametric tests were carried out. Nonparametric analysis showed that animals not receiving a seizure did differ according to drug dosage, Dose 4 animals having a much larger ratio score ($p<.001$) than animals in Doses 1, 2 or 3. When oneway analysis of variance was done on each drug dosage, the nonseizure animals scored significantly higher under Doses 2 and 3. The differences in ratio scores were not significant in Dose - and only significant at the $p<.02$ level in Dose 4.

Body Weight

Because the animals were matched for body weight when assigned to groups and also when they were injected with diazepam, no significant seizure or drug dosage effects were found.

Seizure Comparisons

A 2-way(group and sex) analysis of variance was completed on all the seizure components There were no significant differences in any of the seizure phases between the two groups or between the sexes. It can therefore be assumed that age and sex has no effect on the seizure patterns in these rats.

Relationship between Seizures and Other Behavioural Measures

A Pearson correlation matrix was computed between the six seizure measures and the four main dependent measures to discover if any relationships existed. No significant relationships were found.

Discussion

The purpose of this study was to discover if there is an increase in anticonflict behaviour in response to diazepam in animals who have undergone a seizure. The main indicator of the anticonflict effect was the ratio of the grams of food consumed per approach to the food. The results of this study indicate that the anticonflict response in seizured animals is less than that of nonseizured animals, and that the seizured animals ate less food and made fewer approaches to the food than the nonseizured animals. The general activity of the animals was unaffected by a seizure but the higher dosages of diazepam did cause less activity in all animals. The increasing dosages of diazepam also had the effect of increasing food consumption and decreasing approaches and thus, increased ratio scores in both seizured and nonseizured animals.

The testing procedure used in this study was very similar to that used by Britton and Britton(1981), except that the food source was slightly modified. Wet food was used in this study for 2 reasons: 1) a suitable mechanism whereby a food pellet would remain in place and not be pulled away by the rat, could not be installed without damaging the existing apparatus, and 2) simple observation of animals when they eat showed that they alternate between dry food and drinking water. Providing only dry food may inhibit the amount eaten in a 15 minute period.

For this reason, the quantities of food eaten by the animals were greater in many cases than the amounts consumed by rats in Brittons' study. Opposite to what was predicted animals in the seizure group ate less food than their nonseizure counterparts, but there was a gradual increase in food consumption as the diazepam dosage increased in both seizure conditions. One explanation for these unexpected findings in food consumption, could be that the effects of the seizure, regardless of a postseizure habituation period, were debilitating to the point that fatigue and motor instability prevented consumption or even movements toward the food. If this were the case, one would expect a significantly decreased amount of activity in animals that had a seizure, but as noted in the results, there were no significant differences in activity due to seizure condition. What was affected by seizure condition, however, was the number of approaches to the food and this measure is an integral part of the anticonflict effect. Therefore, the reason behind the decreased consumption of food in the seizure group is related to the anticonflict response more than it is related to the debilitating effects of the seizure. Pentylenetetrazol(PTZ) being a CNS stimulant could have also decreased the hunger response in the animals having a seizure. The increasing dosages of diazepam seemed to have had an increasing effect however, which suggests that the drug still mediated some effect. Some research suggests that PTZ and diazepam bind to the same site on the receptor complex but that diazepam has a

greater affinity (Ibba, Mennini & Testa,1985). If this is the case, then as the diazepam dose increases, the effect of the diazepam should also increase, blocking the effects of PTZ at the receptor site in incremental steps. This may account for the dose related findings here.

It could be suspected that the results are confounded by the fact that benzodiazepines have a hyperphagic effect. Benzodiazepines do tend to increase the consumption of food, and the intake of water and salt solutions (Cooper & Estall,1985). These authors outline numerous studies which support this finding. It may have played a role in this experiment but since all animals were placed on the same feeding schedule, this effect would have been distributed amongst all the animals. Also, in the present study, it was the ratio of grams of food consumed per approach that was considered a prime indicator of the benzodiazepine effect, not the hyperphagic effect. Each gram of food consumed has to be considered against the number of approaches made to the food. Food deprivation, used in this study as a motivation to eat, has not been found to affect the behaviour elicited by benzodiazepines as seen in other studies. Iwahara and Iwasaki (1969,cited in Cooper & Estall,1985), found that chlordiazepoxide increased food intake equally in deprived and nondeprived animals. It can therefore be assumed that deprivation did not effect the amount of food consumed by either seizure or nonseizure animals.

As for the number of approaches, the hypothesis was that under the influence of diazepam an animal would find the bright open field less aversive and therefore spend more time in the center of the field eating food. Fewer approaches and more time spent in the center would be expected. Animals experiencing a seizure did make fewer approaches to the food as seen in the results (Fig. 4.). The nonseizure animals made fewer approaches with the increasing drug dosages, a pattern not seen in the seizure group. The seizure X drug dosage interaction that was found would be expected from the hypothesis because a greater effect of diazepam would assume fewer approaches in the seizure group. The lack of a drug effect in the seizured animals may again lie with the PTZ which could have interfered with benzodiazepine binding, or whose stimulant properties could have increased arousal in these animals. The behaviour demonstrated under Dose 4 may have been due to the acute sedative effects of diazepam or to ataxia, but because of the increase in food consumption at these doses, it can be assumed that the anticonflict effect was affecting the behaviour of these animals. It is therefore not surprising to find a significant decrease in activity with increasing dosages of diazepam regardless of the seizure condition. The lack of a seizure effect in the amount of activity may indicate that the occurrence of a seizure did not affect arousal significantly; it did not incapacitate the animal or prevent it from approaching the food. The differences in approaches caused by the seizure

must therefore be due to the anticonflict effect. Therefore, one could postulate that the anticonflict response was present and that the diazepam had an effect, regardless of the arousal state of the animal.

As part of their study, Britton & Britton (1981) exposed a group of rats to the open field containing food for seven days and injected them on the eighth day with diazepam, to show the effects of habituation to the open field. By Day 7 there was a 90% increase in the amount of food eaten, but a 400% increase in their grams per approach score meaning that as time progressed, the animals learned to go directly to the food and made fewer approaches. This ratio score is a more sensitive measurement because small increases in the food consumed and small decreases in the number of approaches show significant changes in the ratio score rapidly. The results (see Fig. 6) for the ratio score were basically the same as for the other measures, in that the seizure group had lower scores throughout the increasing drug doses of diazepam; an interaction between seizure and dosages was also found. No increased anticonflict effect was demonstrated in the seizure animals as was predicted. The reasons for this finding are speculated in the following discussion.

Since direct study of the changes at the physiological level (ie. changes in receptors) was not possible, this

researcher was basing the hypothesis on the results of previous research that suggests both electrical seizures and chemically induced seizures result in increased numbers of benzodiazepine receptors. PTZ was chosen as the convulsant in this case because of its availability and because pilot studies had established a dose which would cause a rat to have a full tonic/clonic seizure, and return to normal behavioural activity within a short time. Yet even though the animals appeared to return to normal, there may have been seizure activity going on within their brains. The importance of the seizure is a topic of debate. Some researchers have found that kindling will increase the numbers of receptors (Tietz, Gomez & Berman, 1985), while others have found no increase with just kindling (McNamara et al.,1980; Lal et al., 1981) and stress the importance of generalized seizures to cause the increase in binding sites. The precise action of PTZ is not clear although it is known to not block pre or post synaptic inhibition. Franz (1980) suggests that increased extracellular potassium, caused by PTZ, could cause the extreme excitation of CNS cells. Benzodiazepines are one of the substances known to prevent the PTZ seizure, which could relate to the previously mentioned study which found that these two substances occupy the same site on the receptor. Pellow (1985) reviews the research done on the anti-PTZ actions of benzodiazepines. This knowledge of PTZ-benzodiazepine interactions sheds light on the results of this study in that there may have been an interaction of both drugs at the receptor site such that:1) the diazepam suppressed the PTZ effect; 2) the PTZ influenced the diazepam action; and 3) a new action occurs involving some other substance. The

fact that the anticonvulsant effects were not increased in the seizure group could mean that probably one behavioural convulsion was not sufficient to increase the number of receptors and therefore the diazepam did not have more sites on which to bind. The findings could also be a result of an insufficient mechanism by which to gauge the effects; the changes occurring at the receptor level may not be observable behaviourally because the changes are subtle. A concern in this study, as well, is the method used to determine the efficacy of the diazepam. The anxiolytic effect of the diazepam was the action that was chosen but another action, the sedative effect, could have influenced the results substancially. Some researchers (Britton & Britton, 1981) suggest that these sedative effects are secondary to the anxiolytic effects, based on the finding that with prolonged use, the sedative effects are attenuated while the anxiolytic effects persist. The number of receptors may have increased with the seizure but what was seen then was an increased sedative effect rather than an anxiolytic effect.

The mechanisms of seizures and their interactions with neurochemical systems and overt behaviour are relatively unknown. There are many discrepancies in various areas. For instance, there are differences between the actions and consequences of electroconvulsive shock and chemically induced seizures that have not been explained. There are two distinct

types of benzodiazepine receptors and three defined groups of substances that interact with them (namely agonists, antagonists and inverse agonists) but how they interact or even what their functions are has yet to be discovered. In addition to benzodiazepine receptors, there may be other substances mediating the observed effects in this study. The search for endogenous ligands for benzodiazepines has come upon a polypeptide called the diazepam binding inhibitor (Alho,1985) which provides yet another possible explanation of anxiety and seizure activity. As well, an anticonvulsant substance has recently been found in the cerebrospinal fluid of rats that have had a seizure (Tortella & Long,1985). When this substa is injected into recipient rats who are then given a convulsa the seizure threshold increases considerably. The endogenous opioid system is suspect because of the ability of naloxone to attenuate the effect, but other systems cannot be ruled out. This substance may have been involved at the receptor sites in this experiment, perhaps attenuating the effects of diazepam or conversely, having an agonistic effect with the PTZ. Further research is needed to find out more about benzodiazepine sites and their suspected endogenous substrates.

What has been attempted in this study of behavioural changes associated with suspected physiological changes, has also been attempted by other researchers. In a similar study to this one, Katz and Schmaltz (1980) investigated the interaction

between morphine sulfate and electroconvulsive shock (ECT). It was hypothesized that ECT would produce behavioural tolerance to the activating effects of morphine, similar to those shown by morphine itself. Results showed that animals given morphine alone showed increased activity for the whole testing period but that ECT significantly decreased this drug induced activation. A study by Shepard & Broadhurst(1982) looked at the hyponeophagic effects of the interaction of amphetamine and diazepam in rats. The measures used were eating latency and amount of food eaten. In the higher drug dosage of diazepam(10mg/kg.) amphetamine reduced the eating latencies but significantly increased the amount of food eaten in a 10 minute period. These authors explain their results in terms of an arousal hypothesis. Refer to Fig. 7. They used hyponeophagia as the 'conflict' paradigm to test the effects of the benzodiazepines and predicted that the same dose of diazepam could elicit opposite effects depending on the state of the animal. Stimulants given alone would tend to increase eating latency and decrease the amount of food eaten. When the rats were given amphetamine and then diazepam, the lower doses of diazepam failed to attenuate the stimulant action but the larger dose reversed the effects of the stimulant. The authors restrict this explanation to hyponeophagia but the same model could be applied to the present paradigm. The stimulant (PTZ) generally decreased the amount of food eaten compared to nonseizured animals,but increases occurred with higher diazepam

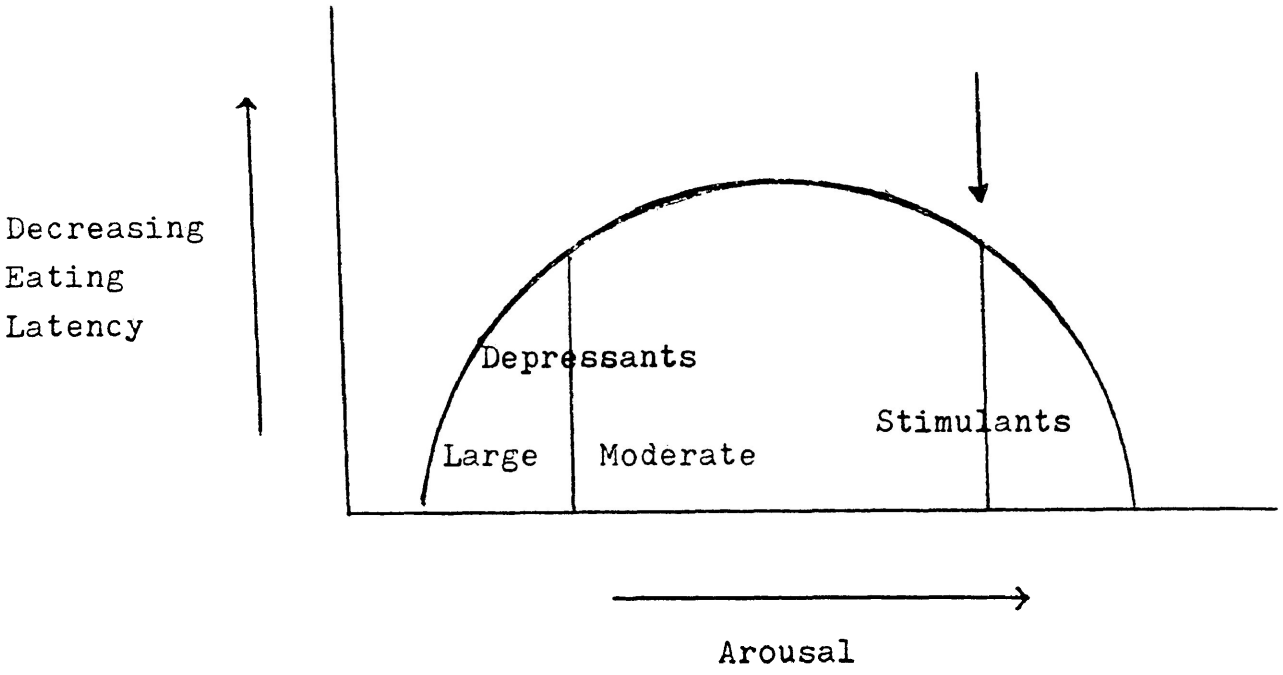

Fig. 7. Arousal hypothesis of hyponeophagia. The descending arrow indicates the suggested, supra-optimal, position on the arousal curve of placebo-injected subjects, and the area to the right of the line beneath this arrow illustrates the increased eating latency induced by stimulants ..... The area between the vertical lines shows the reduced eating latencies observed in response to moderate doses of "depressant" drugs such as those of diazepam....(From Shepard & Broadhurst, 1982, p. 369.)

levels. It is interesting to compare the approaches measure in this study to latency to eating in Shepard & Broadhurst's study. They found that animals given only diazepam showed decreases in latency with increasing doses (0mg/kg, 1mg/kg, 10mg/kg) of diazepam and that animals given both diazepam and amphetamine showed decreasing latencies (opposite to the expected increase amphetamine would cause). In the present study, the nonseizure animals did show the similar gradual decrease in approaches with increasing dosages of diazepam . In the seizure animals the stimulant action of the PTZ was overcome by the diazepam and the same general decrease in approaches occurred although less dramatically than the nonseizured animals.

The reason for mentioning these two studies is that they have similarities to the present one. These studies measure different things but have fundamentally the same purpose in that the researchers looked at the behavioural effects of combining two opposite states within the same animal. All three are examining "stimulant" and "depressant" systems to see which effect dominates in the animals' concommitant behaviour. The results vary depending on the strength of the drugs and treatments used. For instance, in Shepard & Broadhurst's study (1982), they found that animals who received 10mg of diazepam after receiving amphetamine ate more food than animals who got only diazepam. In the present study the animals who had the seizure (ie. equating it to amphetamine) always ate less than

nonseizure animals but did show increases with the diazepam doses. More studies need to be done that replicate the present research to see consistent effects and make generalizations about the effects.

Future research should center around three basic areas of investigation. First, there is a lack of behaviour related studies in this area. Much literature is available on the physiological and biochemical aspects of receptors, but few try to relate the receptor changes to behaviour. Second, the mechanisms of electroconvulsive shock and drug induced seizures need to be studied in terms of their effects on behaviour as well as on brain functioning. If different neurochemicals are released with each type of seizure it may give us more information about the pathology of epilepsy. And third, future studies should examine more closely the effects of various drugs in epileptic humans or those who undergo ECT. If their response to drugs, such as diazepam is different, perhaps it can lead to increased understanding of the illness mechanism or also the other actions of the drug itself. It is common for patients who receive ECT to also be prescribed several drugs and often this chemotherapy involves many neuroleptics. If ECT changes brain physiology, it may be entirely inappropriate to administer certain drugs after ECT. The only way to really determine clear effects is to study the behaviour of these people under varying conditions.

In conclusion, although an increased anticonflict effect was not found in animals after a seizure, the results of this study show that there is a change in response to diazepam after a seizure. The changes seen may or may not be related to changes in brain chemistry. Future research will hopefully determine exactly at what level these behaviour changes are effected.

References

Alho, H.,Costa, E.,Ferrero, P.,Fujimoto, M.,Cosenza-Murphy, D. & Guidotti, A. (1985) Diazepam binding inhibitor: A neuropeptide located in selected neuronal populations. Science 229, 179-182.

Baldessarini, R.(1980) Drugs and the treatment of psychiatric disorders. In A. Goodman Gilman, L.S. Goodman & A. Gilman (Eds) The Pharmacological Basis of Therapeutics. 6th ed. New York: MacMillan Publishing Co. Inc. pp.391-447.

Barr, G.A & Lithgow, T. (1983) Effect of age on benzodiazepine induced behavioral convulsions in rats. Nature 302, 431-432.

Bloom, F. E.(1980) Neurohumoral transmission and the central nervous system. In A. Goodman Gilman, L.S. Goodman & A. Gilman.(Eds) The Pharmacological Basis of Therapeutics. New York: MacMillan Publishing Co.Inc. pp. 235-257.

Braestrup, C. & Neilson, M. (1978) Ontogenetic development of bezodiazepine receptors in the rat brain. Brain Research, 174, 170-173.

Braestrup, C. & Neilsen, M. (1981) Modulation of benzodiazepine receptors. In B. Angrist et al.(Eds.) Recent Advances in Neuropharmacology. New York: Pergamon Press.

Britton, D. K. & Britton, K. Thatcher.(1981) A sensitive open field measure of anxiolytic drug activity.

Pharmacology, Biochemistry and Behavior 15, 577-582.

Cook, L. (1982) Generality of the effects of diazepam on operant behavior in animals and man. In E. Usdin et al. (Eds) Pharmacology of Benzodiazepines. London: MacMillan Press.

Cooper, S J. & Estall, L. B. (1985) Behavioural pharmacology of food, water and salt intake in relation to drug actions at benzodiazepine receptors. Neuroscience & Biobehavioral Reviews 9, 5-19.

Coyle, J. & Enna, S. (1976) Neurochemical aspects of the ontogenesis of GABAergic neurons in the rat brain. Brain Research 111, 119-133.

File, S.(1981) Animal tests of anxiety. In B. Angrist et al. (Eds) Recent Advances in Neuropharmacology. New York: Pergamon Press.

File, S.E. & Vellucci, S.V. (1979) Behavioral and biochemical measures of stress in hooded rats from different sources. Physiology & Behavior, 22, 31-35.

File, S.E. & Tucker, J.C. (1984) Lasting behavioral effects after treating rats with CGS 8216 on postnatal Day 9 to 21. Physiology and Behavior 32, 351-355.

Franz, D. N. (1980) Central nervous system stimulants. In A.G. Gilman, L.S. Goodman & A. Gilman. The Pharmacological Basis of Therapeutics. (6th ed.) MacMillian Publishing Co. Inc. New York. 585-591.

Gallager, D. W.;Mallorga, P.;Thomas, J. W. & Tallman, J.F.

(1980) GABA-benzodiazepine interactions: physiological, pharmacological and developmental aspects. Federation Proceedings 39, 3043-3049.

Gallager, D.W. & Mallorga, P. (1980) Diphenylhydantoin: pre and postnatal administration alters diazepam binding in developing rat cerebral cortex. Science 208, 64-66.

Gallager, D.(1982) Prenatal exposure to a benzodiazepine agonist, antagonist and electroshock: Effects on postnatal development of benzodiazepine binding sites and seizure threshold. In E. Usdin et al.(Eds) Pharmacology of Benzodiazepines.London: MacMillan Press.

Gentsch, C., Lichtsteiner, M. & Feer, H. (1981) H-Diazepam binding in Roman High and Low Avoidance Rats. Experientia 37, 1315-1316.

Gilman, A. Goodman; Goodman, L.S.; & Gilman, A. (Eds) (1980) The Pharmacological Basis of Therapeutics (6th ed.), New York: MacMillan Publishing Co. Inc.

Govoni, L. L. & Hayes, J. E. (1978) Drugs and Nursing Implications. New York: Appleton and Century-Crofts.

Haefely, W.; Pieri, L.; Polc, P. & Schaffner, R. (1975) Possible involvement of GABA in the central actions of benzodiazepines. In E. Costa & P. Greengard (Eds) Mechanisms of Action of Benzodiazepines. New York: Raven Press.

Haefely, W. (1983) The biological basis of benzodiazepine actions.

Journal of Psychoactive Drugs 15(1-2), 19-39.

Hollister, L. E. (1982) Pharmacology and clinical use of benzodiazepines. In E. Usdin et al.(Eds) Pharmacology of Benzodiazepines London: MacMillan Press.

Hollister, L.E. (1983) The pre-benzodiazepine era. Journal of Psychoactive Drugs 15(1-2), 9-13.

Katz, R.J. & Schmaltz, K. (1980) Reduction in opiate activation after chronic electroconvulsive shock:Possible role for endorphins in the behavioral effects of convulsive shock treatment. Neuroscience Letters, 19, (1), 85-88.

Ibba, M.; Mennini, T.; & Testa, R. (1985) Enhancement of diazepam activities induced by denzimol in mice. Pharmacological Research Communications 17(1), 95-103.

Lader, M. (1981) Introduction: clinical aspects. In E. Usdin et al. (Eds) Recent Advances in Neuropsychopharmacology. New York: Pergamon Press.

Lal, H.; Mann, P.A.; Sherman, G.T. & Lippa, A.S. (1981) Effects of acute and chronic pentylenetetrazol treatment on benzodiazepine and cholinergic receptor binding in rat brain. European Journal of Pharmacology 75, 115-119.

Lee, H.K.;Dunwiddie, T.V. & Hoffer, B.J.(1979) Interaction of diazepam with synaptic transmission in the in vitro rat hippocampus. Naunyn Schmeidebergs Archives of Pharmacology 309, 131-136.

Linnoila, M. (1983) Benzodiazepines and performance. In E. Costa (Ed) The Benzodiazepines: From Molecular Biology to Clinical Practice. New York: Raven Press.

Lippa, A.S.; Beer, B.; Sano, M.; Vogel, R.A. & Meyerson, L.R. (1981) Differential ontogeny of Type 1 and Type 2 benzodiazepine receptors. Life Sciences 28,2343-2347.

MacDonald, R. & Barker, J.L. (1978) Benzodiazepines specifically modulate GABA-mediated postsynaptic inhibition in the cultured mammalian neurons. Nature 271, 563-564.

McNamara, J. O.; Peper, A. M. & Patrone, V. (1980) Repeated seizures induce long-term increase in hippocampal benzodiazepine receptors. Proceedings of the National Academy of Science. 77, 3029-3032.

Mennini, T. & Garattini, S. Minireview: Benzodiazepine receptors: orrelation with pharmacological responses in living animals. Life Sciences 31, 2025-2035.

Mohler, H. & Okada, T. (1977) Benzodiazepine receptor: demonstration in the central nervous system. Science 198, 849-851.

Mohler, H. & Richards, J.G. (1983) Benzodiazepine receptors in the central nervous system. In E. Costa (Ed) The Benzodiazepines: From Molecular Biology to Clinical Practice. New York: Raven Press.

Paul, S. M. & Skolnick, P. (1978) Rapid changes in brain benzodiazepine receptors after experimental seizures. Science 202, 892-893.

Paul, S. M. & Snolnick, P. (1982) Comparative neuropharmacology

of antianxiety drugs. Pharmacology, Biochemistry and Behavior 17, 37-41.

Pellow, S. (1985) Can drug effects on anxiety and convulsions be separated? Neuroscience & Biobehavioral Reviews 9, 55-73.

Pert, C.B. & Snyder, S.H. (1973) Opiate receptor: demonstration in nervous tissue. Science 197, 1011-1014.

Polc, P. & Haefely, W. (1976) Effects of two benzodiazepines, phenobarbitone, and baclofen on synaptic transmission in the cat cuneate nucleus. Nauyn-Schmeideberg's Archives of Pharmacology 294, 121-131.

Raabe, W. & Gumnit, R.J. (1977) Anticonvulsant action of diazepam increase of cortical postsynaptic inhibition. Epilepsia 18 (1), 117-120.

Rall, T. W. & Schleifer, L. R.(1980) Drugs effective in the therapy of the epilepsies. In A. Goodman Gilman, L.S. Goodman, & A. Gilman.(Eds) The Pharmacological Basis of Therapeutics. 6th ed. New York: MacMillan Press Co. Inc.

Ramsey, J.M. (1982) Basic Pathophysiology: Modern Stress and the Disease Process. Menlo Park: Addison-Wesley Publishing Company. p.367.

Rickels, K. (1982) Benzodiazepines in the treatment of anxiety. In E. Usdin et al.(Eds) Pharmacology of Benzodiazepines. London: MacMillam Press. pp 37-44.

Robertson, H.A.; Martin, I.L. and Candy, J.M. (1978) Differences

in benzodiazepine receptor binding in Maudsley Reactive and Maudsley Non-Reactive rats. European Journal of Pharmacology, 50, 455-457.

Robertson, H.A. (1979) Benzodiazepine receptors in "Emotional" and "Non-Emotional" mice: Comparison of four strains. European Journal of Pharmacology, 56, 163-166.

Satinder, K.P. & Hill, K.D. (1974) Effects of genotype and postnatal experience on activity, avoidance, shock threshold, and open field behavior of rats. Journal of Comparative and Physiological Psychology 86, 364-374.

Satinder, K.P. (1980) Genetically heterogenous and selected lines of rats: Behavioral and reproductive comparison. Behavior Genetics 10, 191-200.

Schmidt, R.F.; Vogel,E. & Zimmermann, M. (1967) Die Wirkung von Diazepam auf die prasynaptische Hemmung und andere Ruckenmarksreflexe. Naunyn-Schmeideberg's Archives of Pharmacology 258, 69-82.

Shepard, R.A. & Broadhurst, P.L. (1982) Hyponeophagia and arousal in rats: Effects of diazepam, 5 Methoxy-N,N-dimethyltryptamine, d-amphetamine & food deprivation. Psychopharmacology 78, 368-272.

Squires, R.F. & Braestrup, C. (1977) Benzodiazepine receptors in the rat brain. Nature 266, 732-734.

Stein, L. (1982) Benzodiazepines and behavioral disinhibition. In E. Usdin et al.(Eds) Pharmacology of Benzodiazepines. London: MacMillan Press Ltd. pp 383-390.

Stein, L.;Wise, C.D. & Belluzzi, J.D. (1975) Effects of benzodiazepines on central serotonergic mechanisms. In E. Costa & P.Greengard (Eds) Mechanism of Action of Benzodiazepines. New York: Raven Press. pp 29-44.

Tietz, E. I., Gomez, F., Berman, Robert.F. (1985) Amygdala kindled seizure stage is related to altered benzodiazepine binding site density. Life Sciences 36, 183-190.

Tortella, F.C. & Long, J.B. (1985) Endogenous anticonvulsant substance in rat cerebrospinal fluid after a generalized seizure. Science 228, 1106-1108.

Vogel, J.R.; Beer, B. & Clody, D. E. (1971) A simple and reliable conflict procedure for testing anti-anxiety agents. Psychopharmacologia 21, 1-7.