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## The Effects of Alprazolam and Triazolam on Two Methods of Induced Aggression in Rats

Howard Kiel Plummer

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THE EFFECTS OF ALPRAZOLAM AND TRIAZOLAM ON  
TWO METHODS OF INDUCED AGGRESSION IN RATS

by

Howard Kiel Plummer III

A Thesis  
Submitted to the  
Faculty of The Graduate College  
in partial fulfillment of the  
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Department of Biology

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THE EFFECTS OF ALPRAZOLAM AND TRIAZOLAM ON  
TWO METHODS OF INDUCED AGGRESSION IN RATS

Howard Kiel Plummer III, M.A.

Western Michigan University, 1984

Two new benzodiazepine tranquilizers, alprazolam and triazolam were used to try to reduce aggression in rats produced by isolation or drugs. Both reduced elements of isolation-induced aggression, and triazolam was more potent. Little reduction was found in drug-induced aggression by either tranquilizer. It is unknown why aggression was not reduced for the drug-induced method, but it is possible that a synergism existed between the two methods of inducing aggression, since the same rats were used for both methods. Different rats were used for each tranquilizer. Reduction in aggression for the isolated rats seems consistent with results for other benzodiazepine tranquilizers.

#### ACKNOWLEDGEMENTS

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Special thanks go to my mother, for suggesting the two drugs to work with, and providing some helpful information.

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Howard Kiel Plummer III

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WESTERN MICHIGAN UNIVERSITY

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## INTRODUCTION

Aggressive behavior is important for survival of an animal species. This behavior is often studied for its reactions to drugs because it is regulated by structures of the central nervous system. The hypothalamus and the other structures of this system are sensitive to the effects of drugs (Miczek and Barry, 1976).

Aggression is a damage oriented set of behaviors, where the responses of one organism to another results in destruction, dislocation or disfigurement. There are multiple causes for aggression. Aggressive behavior can result either from strong environmental stimuli, or from the results of these stimuli (Hutchinson, Emley and Krasnegor, 1976).

According to Moyer (1968) there are several kinds of aggression, each one having its own neural and endocrine basis. These types of aggression include predatory, fear induced, irritable, territorial, maternal, instrumental and inter-male.

During intraspecific fighting situations in rodents one animal will assume dominance, while the other one will be submissive. These behaviors are highly stereotyped, and will be discussed later. An interspecific aggressive behavior that is studied in rats is the mouse-killing response. This is found in 10 to 30 percent of all laboratory rats (Miczek and Barry, 1976).

## Inducing Aggression

Researchers have used various methods to induce aggressive behavior. The following techniques are used because they show behaviors that resemble hostile or aggressive behaviors in man. It is, therefore, thought that the results of this animal experimentation can be transferred to man (Cook and Kelleher, 1963).

There are several methods for inducing aggressive behavior in mice and rats. The most frequently used method is isolation. Valzelli (1967) stated that isolation is a social situation that can induce a high degree of excitability and then aggressiveness. Barnett and Taber (1971) state that virtually all tests for aggression can be done with either the mice or rats, even though there are certain qualitative differences in response to certain drugs. According to Miczek and Barry (1976) after a period of isolation, when two animals meet, they will usually fight. However, this method of inducing aggression has its problems. The isolation period should not be too long or an isolation syndrome will develop, and results of any aggression will be biased. Leavitt (1974) states that after isolation mice become hypersensitive to all stimuli.

Valzelli (1973a) stated that isolation has been widely described to give rise to strong aggressive behavior in many animal species. Changes in mice include variations in peripheral,

behavioral and neurochemical functions. Valzelli and Garattini (1972) stated that rats isolated for six weeks developed three different patterns of behavior towards mice-friendly, muricide or indifferent.

Rats isolated for long periods of time develop certain behavioral and structural changes. They become nervous and develop caudal dermatitis. After thirteen weeks of isolation, rats have heavier adrenals and thyroids, but have lighter spleens and thymus compared to non-isolate rats. Rats also have decreases in numbers of circulating lymphocytes, and the females showed an increased response to adrenocorticotrophic hormone (ACTH) (Hatch et al., 1965; Hatch et al., 1963).

A second similar method for producing aggression is by introducing a stranger into an animal's home environment. This also has problems. After several introductions of the same stranger animal, familiarity will develop, and aggression diminishes (Miczek and Barry, 1976).

Pain-induced aggression is a third method. Usually an electric shock is delivered through a grid floor, causing the animals to assume an aggressive posture. Pain is a powerful stimulus for aggression. The problem with this method is that it is not clear whether or not shock-induced aggression resembles naturally occurring aggression (Miczek and Barry, 1976).

Two other methods for evoking aggression in rodents are brain lesions, and drug-induced aggression. Aggression can be induced by applying a brief electric shock to structures in limbic,

diencephalic and mesencephalic portions of the brain. These parts of the brain may be cut out of the experimental animal to provide the same results. Although many conflicting results exist, certain drugs can induce aggression. Among these drugs are tetrahydrocannabinol (THC), lysergic acid diethylamide (LSD), parachloroamphetamine (PCA) and parachlorophenylalanine (PCPA). Both these methods induce aggression by depletion of cerebral serotonin. There are other methods that can be used for developing aggressive behavior, but are more psychological (i.e., conditioning) than biological, and will not be covered (Miczek and Barry, 1976; Sheard, 1976).

Contrary to the majority of published data, Krsiak and Steinberg (1969) state that it is uncommon for drugs to have any effect on aggressive behavior. They do, however, say that in some conditions LSD and mescaline can inhibit aggressive behavior which has been induced by methods such as isolation or pain.

#### Measuring Aggression

Several methods for recording aggressive behavior have been devised. These range from rating scales to ethological analysis of specific behavior patterns. Rating scales for one behavior have often been used, but are not very good. Measuring only one behavior can often misrepresent events. Some drugs have been shown to effect one aspect of aggressive behavior, but will not effect another. Rating scales using a variety of behaviors are much



better. Another method of scoring aggressive behavior is on a scale from zero to 100, but this method is problematic. (Miczek and Barry, 1976; Valzelli, 1967).

According to Norton (1957), the amount of times an item occurs is related to the intensity of internal stimulus necessary to produce it. This is another reason rating scales consisting of a variety of behaviors are better. According to Grant and Mackintosh (1963) there are common postures that all rodents show for social behaviors, including behaviors for aggression. These behaviors include introductory acts, flight behavior, submissive behaviors and aggression.

Yen, Stranger and Millman (1959) found other factors important while working with aggressive behaviors. Sex of the experimental animal was found to be important, because male mice and rats are much more aggressive than female. Younger rodents were found to fight more than older ones. Also important were strain or breed, and period of isolation. Leavitt (1974) found that rats are attracted more to moving objects than to inanimate objects. By reducing an animal's activity by drugs, its ability to change the other animal's behavior will be decreased. The rat was the animal chosen for the experimentation in this thesis.

#### Methods Used in This Thesis

Isolation-induced aggressive behavior is a fairly simple technique that has been used by many researchers. The isolation

period should be at least three weeks in length. According to Valzelli and Garattini (1972) prolonged isolation in mice not only produces strongly aggressive behavior, but a decrease in five-hydroxyindole-acetic acid (5-HIAA) content plus a decrease of serotonin (5-HT) turnover rate. These biochemical changes only occurred in the mice which became aggressive after isolation, and did not appear in non-aggressive animals. Therefore, this could be a possible mechanism of action in isolation. Garattini, Giacalone and Valzelli (1967) also found a possible relationship between behavioral changes and 5-HT turnover.

As stated previously, Valzelli and Garattini (1972) found three types of rat behavior towards mice after isolation-muricidal, friendly or indifferent. The serotonergic turnover rate increased in friendly rats and decreased in muricidal and indifferent rats. Isolation is an interesting technique for studying psychotropic drugs, because of this possible relationship between neurochemical and behavioral changes in rats.

Drug-elicited aggression is another fairly simple technique for producing aggressive behavior. The drug for this experiment was parachloroamphetamine (PCA). PCA is a known serotonin depletor, which usually causes aggression. According to Gallus et al. (1982), after parenteral PCA injection, serotonin is released from central nervous system neurons. This leads to a serotonin-mediated abnormal motor syndrome, and steady declines in serotonin and five-hydroxyindole acetic acid levels, which persist for several months.

Sanders-Bush and Steranka (1978) have found that PCA in rats caused a complex pattern of changes in brain amines and behavior. The decrease in 5-HT and its principal metabolite, 5-HIAA is due to many mechanisms. These include the release of 5-HT, inhibition of its reuptake, and synthesis. Lassen (1974) also found a prolonged and simultaneous decrease in cerebral serotonin.

Scheel-Kruger (1972) found that PCA influenced catecholamines and stimulated serotonin mechanisms. Strada and Sulser (1971) also support the view that the central actions of PCA may be mediated through the release of stored catecholamines. Sanders-Bush (1976) found that the levels of 5-HT and 5-HIAA, plus activity of tryptophan hydrolase decrease rapidly, and remain for at least four months after a single dose of 10 mg/kg of PCA. With a dose of 5 mg/kg the metabolites remain reduced for only two weeks.

Sanders-Bush (1976) also found that PCA has a selective neurotoxic effect on the 5-HT neurons in the brain. They remain damaged for several months after treatment with the drug. Although PCA is a relatively inert compound, there is formation in the brain of a highly reactive derivative which can lead to structural damage. According to Sheard (1976) it is reasonable to think that this damage can lead to specific behavioral changes. Methods that inhibit brain 5-HT or lower the levels of it in the brain also decrease the pain threshold in rats.

PCA has early effects on rat behavior that are unrelated to its later serotonergic lowering effects. These effects according to Growdon (1977) include postural abnormalities, tremor, myoclonus,

salivation and piloerection. These effects begin five minutes after injection and last from sixty to ninety minutes. Another effect found by Schell-Kruger (1972) was a long-lasting compulsive forward walking activity right after injection.

According to Sanders-Bush (1976) PCA disappears from the brain of rats fairly soon. During the first sixteen hours, the drug declines with a half-life of six to ten hours. It seems that the long term effects of PCA are unrelated to the presence of the drug.

The effects of PCA on aggression follow this double mechanism of action. Fifteen to thirty minutes after injection, there is a decrease in shock-elicited aggression, followed by a gradual return over the next one to two hours. After that, there is an increase in fighting, not only in frequency, but in intensity (Sanders-Bush, 1976).

There are other drugs that have very similar effects on serotonin. Parachlorophenylalanine has almost the same results as PCA. According to Brody (1970) it decreases serotonin and increases shock intensity needed in order to get a jumping response. Tenen (1967) found that rats reduced their activity and were more irritable. Large doses of parachlorophenylalanine induced mouse killing aggression towards rat pups according to Miczek et al. (1975). They think the findings suggest 5-HT depletion might facilitate non-specific killing responses. Sewell et al. (1982) found that the drug increases shock-induced aggression, but that this result is not selective for aggression.

Delta-nine tetrahydrocannabinol also has effects on serotonin

in the brain. Sofia, Dixit and Barry (1971) found that synthesis rate of 5-HT was reduced in rats after treatment. However, whole brain levels of 5-HT were elevated. Holtzman et al. (1969) found a slight increase in concentration of 5-HT after treatment with tetrahydrocannabinol. They also found a decrease in norepinephrine at low doses and an increase after high doses. The authors noted that these changes did not correspond to those that have been observed with other psychotropic drugs.

Stein and Wise (1974) state that norepinephrine and serotonin exert opposing effects on aggressive behavior. Ellison and Bresler (1974) found that alternation in brain catecholamines, norepinephrine or serotonin may be correlated with changes in emotion, drive or mood. Changes in brain amines also cause rats to be more aggressive when shocked and highly reactive to environmental stimuli. Bliss, Ailion and Zwanziger (1968) found that the stress of foot shock in rats reduces the levels of brain norepinephrine, but does not effect serotonin. It is interesting to note that all the drugs and mechanisms described in this thesis have some effect on serotonin.

#### Tranquilizers Used in Aggression Research

For the thesis research, two new tranquilizers from The Upjohn Co. were chosen. These tranquilizers are new triazolobenzodiazepines, which are members of the class of drugs known as antianxiety drugs, which are central nervous system depressants. These two drugs are

alprazolam and triazolam. Although rats were used in this experiment, the results should be similar to those found in mice.

Central nervous system depressants have been shown to reduce aggression. These drugs, also known as the sedative-hypnotics, include barbiturates, non-barbiturate hypnotics, antianxiety agents or minor tranquilizers, alcohols or anesthetic gases, and anti-psychotic agents or major tranquilizers.

Tranquilizers alter emotional responses of the organism. These drugs produce mainly behavioral changes, and not physiological changes. Major tranquilizers are more potent than minor tranquilizers. According to Scriabine and Blake (1962) chlorpromazine (major) is thirteen times more potent than chlordiazepoxide (minor). Burn and Hobbs (1958), Salustiano, Hoshino and Carlini (1966), Haley (1957) and Silverman (1965) have all found that chlorpromazine reduced aggression in rodents in all methods tested. Much less consistent results have been found for minor tranquilizers.

Minor tranquilizers include the carbamates and the benzodiazepines. Anti-anxiety agents have replaced barbiturates in treatment of anxiety in people. Carbamates, such as meprobamate, are more sedative than benzodiazepines. Benzodiazepines are more selective in treating anxiety.

According to Julien (1981) normal doses of anti-anxiety agents act on arousal centers in the brain. The two centers, located in the brainstem and the midbrain, are important for the maintenance

of behavioral arousal. Babbini, Gaiardi and Bartoletti (1979) state that the sedative effect often invokes a different neural mechanism than the antianxiety effect of benzodiazepines. It is not surprising, therefore, that these drugs have different results on aggressive tests.

Chlordiazepoxide was synthesized in 1955 at Roche laboratories. Studies on the effects of its structure found that it had sedative-hypnotic effects similar to meprobamate. It was approved for marketing in 1960. As early as 1960, chlordiazepoxide was shown to have a taming effect on monkeys and wild zoo animals. Chlordiazepoxide was the first developed of this new class of psychotropic drugs that show primarily anti-anxiety effects in humans. In animals, these effects can be correlated to include taming behavior, plus attenuation of fear in avoidance and conflict situations (Valzelli 1973b; Greenblatt and Shader 1974).

Diazepam was synthesized in 1959, approved in 1963 and is similar in effects to chlordiazepoxide, but more potent. Oxazepam was marketed in 1969, while flurazepam has been available since 1970. In 1972 clorazepate dipotassium was marketed in the United States (Greenblatt and Shader, 1974). Alprazolam was marketed in August, 1981, and triazolam in December, 1982.

According to Christmas and Maxwell (1970) benzodiazepines are interesting because they lower shock-induced aggression in mice and rats at low doses. These doses are usually far removed from those causing a reduction of locomotor activity.

According to Greenblatt and Shader (1974) benzodiazepines have

variable effects on the aggressive behaviors in animals. These effects usually depend on species, strain, sex, social environment, drug dosage and route of administration. The method used to bring about aggressive behavior also affects the results. In most studies, aggression and hostility are reduced, but under certain circumstances benzodiazepines can stimulate hostile behavior.

DeMascio (1973) investigated chlordiazepoxide and other benzodiazepines including diazepam, oxazepam and nitrazepam using a variety of techniques. Aggression induced by septal lesions was most resistant to the effects of benzodiazepines. Shock or isolation induced aggression was reduced only at high dose levels. Chemically or drug induced aggression was either reduced or increased depending on the chemical used. Induced aggression was most easily reduced, while naturally occurring aggression was either not affected or was increased.

According to Valzelli (1973b) it is very difficult to show psychoactive drugs as antiaggressive because of the different mechanisms of these aggressive behaviors in animals. Conflicting results are often found using benzodiazepine derivatives in animals. However, for induced aggression types, including isolation, aggression seemed to be reduced, while naturally occurring aggression was unaffected.

Benzodiazepines also have muscle-relaxing effects in addition to their possible effects on aggression. These effects are clearly unrelated. Valzelli, Ghezzi and Bernasconi (1971) think that differing results of benzodiazepine drugs on aggression may be due



to changes of biochemical and emotional characteristics of experimental animals. These are usually induced by an alternation of their normal social environment. According to Methot and Deutsch (1984) in addition to reducing conflict, benzodiazepines increase appetite.

Various other authors have also found contradictory results with benzodiazepines. Some have found increases in aggression, while others have found decreases. Malick, Sofia and Goldberg (1969) found that chlordiazepoxide and diazepam were inhibitory in three lesion-induced models of aggression in the rat. Dantler (1977) noted the taming action of these two drugs.

Cook and Kelleher (1963) found that chlordiazepoxide and diazepam reduced aggression produced by septal lesions. Isolation induced aggression was unaffected by chlordiazepoxide, while shock induced aggression was reduced by the drug. These three methods of creating aggressive behavior probably do not involve the same mechanisms, so it is not surprising that there are differences in the results (Cook and Kelleher, 1963).

Leaf et al. (1975) found that chlordiazepoxide and diazepam increased the low rates of mouse-killing observed in rats. The validity of their results is questionable since Horovitz (1966) found muricide inhibited using chlordiazepoxide and diazepam. This latter study has much more valid results.

Other studies have been done using diazepam. Boissier, Grasset and Simon (1968) determined that after a short period of isolation, diazepam and chlorpromazine reduced aggressive behavior in mice.

Fox and Snyder (1969) reported that diazepam treated males exhibited more aggressive encounters and fewer defensive postures during the initial period of grouping male mice together. Malick (1978) stated that diazepam was a selective antagonist of fighting behavior in isolated mice following administration of the drug for five days. Low doses were necessary to produce reduction in isolation-induced aggression following only acute administration.

Chlordiazepoxide has shown similar differing effects on aggression in several other investigations. According to Miczek (1974) chlordiazepoxide changes the attack and threat behaviors in rats in a dose dependent biphasic manner. Low doses of the drug increased aggression, while high doses decreased aggression. The drug also had an affect on submissive and defensive behaviors. Both the immobility reaction and submissive behaviors were prolonged.

Zwirner, Porsolt and Loew (1975) tested male mice with chlordiazepoxide, and showed that in low doses aggressive behavior was increased, while motor activity was not affected. They concluded that higher doses were needed for taming effects. Olivier and VanDalen (1982) showed similar results. Chlordiazepoxide increased both aggression and social activities in both rats and mice.

Quenzer, Feldman and Moore (1974) found that chlordiazepoxide significantly reduced shock-induced aggression, and that chlordiazepoxide was effective in suppressing irritable aggression. In a

fairly poor study by Fox, Tuckosh and Wilcox (1970), chlordiazepoxide increased spontaneous aggression and combat associated mortality. This study is poor and results are questionable since the isolation period was only six days, whereas at least a three week period is normally accepted as necessary to produce aggression by Valzelli (1973a) and others. Another problem with this experiment is that the mice were fed the drug continuously, allowing no regulation of the dose. Thirdly, effects of aggression were measured by mortality of mice on the drug diet versus non-drugged mice. These criticisms suggest these data are not very valid.

Chlordiazepoxide, diazepam, as well as other tranquilizers and antidepressants had various results on aggression in a study by Sofia (1969). Chlordiazepoxide and diazepam reduced aggression produced by shock, but had no effect on septal lesion or isolation induced aggression.

According to Valzelli, Giacalone and Garattini (1967), chlordiazepoxide, diazepam, oxazepam and nitrazepam reduced aggressive behavior in isolated mice. They were using a zero to 100 scoring system. Krsiak (1974) found that singly housed mice fed with diazepam showed more aggressive encounters and fewer defensive postures when they were grouped for forty minutes. Similar results were found for chlordiazepoxide, nitrazepam and flurazepam. Krsiak (1974) concluded that this result was surprising, since benzodiazepines are thought to be selective

inhibitors of aggressive behavior. The same problem might exist with this study as with the Fox, Tuckosh and Wilcox (1970) experiments. Feeding does not allow control of the dosage.

Triazolobenzodiazepines are a fairly new series of compounds that produce effects similar to the known antianxiety agents such as diazepam and chlordiazepoxide. Triazolobenzodiazepines differ from diazepam by addition of a five membered ring at position one and two of the benzodiazepine structure. This produces a product that is more potent than diazepam, and has differences in its activity range (Rudzik, Hester and Friis 1973). Chemical structures of alprazolam and triazolam are given in Figure 1.



Figure 1. Chemical structures of alprazolam and triazolam.  
(Adams, 1979)

Other authors have also found that triazolobenzodiazepines have similar effects to known compounds such as diazepam and chlordiazepoxide. According to Sethy and Harris (1982a) triazolobenzodiazepines such as alprazolam and triazolam were found to be both anxiolytic and hypnotic in man. Nakajima et al. (1971)

found that two new triazolobenzodiazepines D-40TA and D-65MT were more potent than diazepam or nitrazepam in calming rats made aggressive by septal lesions. Rudzik, Hester and Friis (1971) have found that triazolobenzodiazepines antagonize the aggressive behavior produced by foot shock in mice.

Some work has been done with alprazolam and triazolam on aggression and anti-conflict behavior in animals. No work, however, has apparently been done on isolation or drug-induced aggression in rats. Most of the work with these two compounds has been done in other areas, relating to human behavior and drug potency.

Wilson, Phillips and Phillips (1974) found that U-31,889 (alprazolam) was ten times as potent as diazepam. Ueki et al. (1981) discovered that the anticonflict effects of alprazolam were more potent than diazepam and lorazepam in rats. In their study, alprazolam suppressed aggression and muricide by olfactory-bulbectomized rats. Muricide by rats with raphe lesions was also inhibited by alprazolam. Wilson, Phillips and Phillips (1974) concluded that alprazolam probably has an effect on the central serotonergic mechanism.

Reserpine produced depressive effects in animals have been used for screening antidepressant drugs. According to Sethy and Hodges (1982c) this increases beta-adrenergic receptors in the cerebral cortex. This effect is partially blocked by treatment with alprazolam, while diazepam does not effect this. This may be related to the mechanism of action of alprazolam.

According to Castaner and Chatterjee (1976a) alprazolam has a taming effect on aggressive mice and monkeys, and also produces muscle relaxation. Alprazolam was found to be three times as potent as diazepam, and forty times as potent as chlordiazepoxide. About one-tenth the dose of diazepam was necessary to produce central nervous system depression when using alprazolam. Alprazolam appears to be as effective as diazepam in treating anxiety in humans, but lower doses are needed to produce the same effect (Anonymous, 1982).

Similar studies exist for triazolam. Ueki et al. (1978) found that triazolam four to five times as potent as diazepam in reducing aggression produced by either septal lesions or by olfactory bulbectomized rats. Triazolam also reduced muricide in olfactory bulbectomized rats. The drug worked similar to diazepam in inhibiting fighting behavior of long term isolated mice. While the dosage of triazolam was about the same as diazepam, the effect lasted longer.

Gomito (1978) found that conditioned behavior was reduced by both triazolam and diazepam in large doses. Conflict situations produced by shock induced aggression and food reward were reduced by both drugs. The potency of triazolam was ten to fifteen times greater than diazepam. File (1981) reported tolerance to the effects of administration. Acute administration of lorazepam and triazolam reduced exploring behavior in rats. Castner and Chatterjee (1976b) also have determined that triazolam is also more potent than other benzodiazepine drugs.

Kitagawa, Esumi, Kurosawa, Sekine and Yokoshima (1979a)

identified the time necessary for triazolam to become effective by using radioactivity. Peak radioactivity in the blood was reached in rats thirty minutes after i.p. injection, and one hour after oral dosing.

Experiments with shock-induced aggression were done in male albino rats using triazolobenzodiazepines. Taming effects were also studied in Rhesus monkeys. Four different triazolobenzodiazepines were used, including alprazolam and triazolam, and it was discovered alprazolam was about fourteen times more potent than diazepam in reducing shock induced aggression in mice. In monkeys, triazolam was ten times more active than diazepam in inhibiting aggressive behavior. These tranquilizing agents have low toxicity in laboratory animals (Rudzik et al., 1973).

Triazolam was discovered to be more potent than alprazolam in reducing conflict behavior in rats, but only equipotent on reducing aggressive behavior. Most of the triazolobenzodiazepines have the same type of activity as diazepam (Rudzik et al., 1973).

Methods of action of the benzodiazepines including triazolobenzodiazepines have been postulated. According to Wise, Berger and Stein (1972) benzodiazepines while reducing anxiety, also decrease the turnover rate of serotonin in the brain. This was maintained with repeated doses, while a change in norepinephrine turnover underwent tolerance. Tranquilizers may exert their results by reduction of serotonin activity.

Cook and Sepinwall (1975) tested several methods for mechanism

of action of benzodiazepines. They excluded cyclic AMP phosphodiesterase, GABA, or glycine receptors. Partial support was found for serotonin. Quenzer, Feldman and Moore (1974) found oxazepam reduced turnover rates of brain 5-HT. According to Julien (1981) diazepam and clorazepate are metabolized to oxazepam in the body, so it is likely that similar results would be found.

There is also evidence of benzodiazepine receptors in the brain. Using mice, Lippa et al. (1978) found binding of benzodiazepines in cerebellar purkinje cells. Benzodiazepines could exert their antianxiety effects this way. According to Squires and Braestrup (1977) the binding sites of benzodiazepines are distributed unevenly through the brain. The displacement of these receptors correlate with the pharmacological effects predictive of anxiolytic activity.

Braestrup and Squires (1978) found that rat brain tissues contain specific benzodiazepine receptors. These are probably membrane-bound and probably proteins, and are highly specific for benzodiazepines. Triazolobenzodiazepines show high affinities for these receptors. Sethy and Harris (1982b) found that acute administration of alprazolam and diazepam increased the number of these receptors.

Benzodiazepines also have other effects on the brain. Kitagawa, Esumi, Kurosawa, Sekine and Yokoshima (1979b) found that species differences exist in the metabolic pathways of triazolam. According to Sethy and Solomon (1977) benzodiazepines have effects on acetylcholine concentrations in the rat brain. Diazepam,



flurazepam and triazolam significantly increased acetylcholine concentration in the cerebral cortex and striatum. Alprazolam and ketazolam had no effect on the concentration. Benzodiazepines may only have effects on certain areas of the brain.

Benzodiazepines have other effects on the brain. According to DeLorenzo, Burdette and Holderness (1981) diazepam causes a significant inhibition of the calcium-calmodulin protein kinase system in the brain. This was produced by the membrane bound benzodiazepine. The effects of benzodiazepine may be modulated by this system, and this may be one of the benzodiazepine receptors in the brain.

## METHODS

To study aggression in male albino rats, two methods of inducing aggression were used. The rats for the experiments were acquired from the R.G. Sewell Laboratory in the Behavioral Effects of Cancer Therapy, Department of Psychology, Western Michigan University, Kalamazoo, MI 49008.

All rats were adult male albinos, and weighed between 400 and 550 grams. Subject rats had Purina Laboratory Rat Chow and water available ad libitum. They were housed singly in cages 18 by 23 by 18 cm. The cages had solid sides and backs, with wire fronts and bottoms. These cages were placed in a rack manufactured by the Geo. H. Wahmann MFG. Co., Baltimore, MD. The cages were kept in two rows of five each. The colony room had continuous light and was kept at a constant temperature of 21°C.

Forty rats were used in the experiments. Ten rats were used for control, ten rats for each of the two tranquilizers used in this experiment, and ten rats for the vehicle in which the tranquilizers were suspended.

Two methods of eliciting aggression were used, isolation and drug-induced aggression. To begin the experiments, ten rats were isolated in single cages for at least three weeks. Since the sides of the cages were solid, no rat could see another. This method of

isolation was proposed by Yen, Stanger and Millman (1959) and was used in this experiment to determine several parameters.

Since strain, sex, age, and the isolation period were found to be important in this type of induced aggressive behavior, these factors were kept constant. Only male rats of the same strain and approximately five months old were used. Weight was as constant as possible and rats were isolated in the same size cages and for the same period of time.

After the three week period of isolation, rats were placed in the test chamber and observed for various aggressive behaviors. According to Miller and Barry (1960) there are several factors that expediate experimental procedures. One of these factors is careful observation of the animal. The rat to be tested was placed in the experimental chamber twenty minutes prior to the start of the test, and the introduction of the second rat, and then carefully watched.

This twenty minute period was to give the testee the home cage environment. As previously stated by Yen, Stranger and Millman (1959) when a rat is placed in the home cage of another, both rats will fight. Also it has been shown that two rats placed together after isolation will fight.

As recommended by Miller and Barry (1960) different drug doses were given.

The test chamber for the experimentation was 47 by 54 by 31 cm., wire bottomed with the lower half of the sides solid, and the upper half slotted.

The ten rats were placed systematically with one another to test for aggression and submission. The rats were tested consecutively one through ten, and placed with another rat from the opposite row of cages. No rat was placed with the same rat for two consecutive trials. As much as possible, the same rats were not put together. The test lasted for thirty minutes and each instance that a particular aggressive or submissive behavior occurred was noted and recorded. This procedure will be described in greater detail in the tranquilizer section. After this isolation test procedure, the same rats that had been isolated three weeks were prepared for the drug-induced aggression study. They were injected with 10 mg/kg of parachloroamphetamine. All injections were intraperitoneal. The parachloroamphetamine was prepared from d,l-parachloroamphetamine hydrochloride (Sigma Chemical Co., St. Louis, MO) and delivered in an isotonic saline vehicle.

Since Gallus et al. (1982) found that rats had high mortality after parachloroamphetamine injection for the first twenty-four to forty-eight hour period when either handled or in groups, the rats were isolated and not disturbed for a forty-eight hour period. After this period of time, the rats were once again placed in the test chamber and tested for aggressive and submissive behaviors. The same procedure was used as was used for the isolation-induced aggression tests.

After the testing procedure for these control rats, they were discarded and a second set of ten rats were used to discover what effect the vehicle for injecting the two tranquilizers would have

on aggression. The vehicle, a 0.5 percent solution of carboxymethyl cellulose in sterile distilled water, was injected intraperitoneally into the test animal twenty minutes prior to the start of the test.

These rats were also isolated for at least three weeks prior to the start of the experimentation. After injection of the carboxymethyl cellulose, the rat was placed into the experimental chamber. The same procedure described above for the control rats was used again. After testing of all ten rats, they were also injected with parachloroamphetamine, and tested again using the same procedure described above. After testing, all ten rats were discarded.

Alprazolam and triazolam were provided by The Upjohn Co., Kalamazoo, MI 49008. The two triazolobenzodiazepines were dissolved in the 0.5 percent carboxymethyl cellulose solution. Three doses of each drug were made up. The doses of alprazolam used were 0.5, 1.0, and 2.0 mg/kg. For triazolam, 0.25, 0.5, and 1.0 mg/kg doses were used. These were injected into the rats twenty minutes prior to the start of the test, as was the vehicle for these drugs.

Kitagawa, Esumi, Kurosawa, Sekine and Yokoshima (1979a) found peak radioactivity in the blood from marked triazolam thirty minutes after injection. The drugs were injected twenty minutes prior to the start of the test, so that peak effect of the drug occurred during the test.

The procedure used for these rats was the same as in previous

experiments. Two different sets of ten rats were isolated for each of the two drugs; alprazolam and triazolam. After three weeks of isolation, rats were tested for their aggressive and submissive behaviors in the test chamber.

After injection of the lowest of the three drug doses, the rat's fur was marked with black magic marker and placed in the test chamber. Twenty minutes later the second rat was placed in the chamber, and the thirty minute trial began. Behaviors described by Grant and Mackintosh (1963) and further elaborated by Silverman (1965) and Sieber, Frischknecht and Waser (1980) were observed and were marked down each time they occurred. After the test, the rats were returned to their respective cages until the next experiment. All ten rats were tested this way and were placed with one another in the same systematic manner as described before.

The second and third doses were done in the same fashion, using the same rats for all drug doses. After testing the rats at each of the three drug doses, they were injected with parachloroamphetamine and once again tested for all three drug doses of the tranquilizers. All rats were tested using the same procedure as previously. After testing both of the tranquilizers this way, the rats were discarded.

The behaviors observed in this experiment were described by Grant and Mackintosh (1963) and are as follows:

**Aggression:**

Approach - a movement towards the other rat.

Aggressive Posture - The aggressive animal orientates himself

at right angles to and over the other rat.

Aggressive Groom - Nibbling or grooming fur of the other rat.

Threat - Head movement towards the other rat.

Thrust - Moving the whole forefront of the aggressive rat's body towards the other rat.

Attack - Movement rapidly towards the other rat. This behavior resembles Aggressive Posture.

Offensive Upright - Aggressive rat stands on hindlegs while oriented to the other rat.

Offensive Sideways - Aggressive rat approaches the other broadsides.

Chase - Chasing the other rat around the cage.

Bite - Biting the other animal.

#### Flight, Escape, Submission:

Defensive Upright - Rat on hind legs, but his head is not oriented towards the other rat.

Defensive Sideways - Rat presents its side to the other rat, and often has its ventral side towards the other rat.

Submissive - Rat lies on its back.

Retreat - Movement away from the other rat.

Flag - The submissive correlate to threat behavior. Moving the head away from the other rat.

Evade - The submissive correlate to thrust behavior. Moving the forefront of the body away from the other rat.

Crouch - Rat is on all four paws, often has its shoulders

lowered.

Freeze - Rat does not move.

Statistical tests were run on the results of the experiments. All aggressive behaviors were added up and a t-test was run on the combination of behaviors. Each rat was compared to its counterpart rat in the treatments. The t-test was run between the control and the seven treatments, which were carboxymethyl cellulose, and the three doses of each of the two drugs. Submissive behaviors were also summed, and the t-test run between the control and the seven treatments. Results that were found to be significant at the 0.05 level had an analysis of variance and covariance run between the three doses of the drug, to find if increasing dose was significant.

The two different methods of producing aggression were treated differently. Isolation-induced aggression was treated separately from PCA-induced aggression. The t-test was run on both, but the different methods were kept separate. No statistical tests were run between the two methods of inducing aggression.

Statistical tests were also run on the individual behaviors. The behavior of the ten control rats was compared to each of the seven treatments. The t-test was also run on these individual behaviors. Results that were significant were tested by an analysis of variance and covariance.

The statistical tests were run on the Western Michigan University computer using the BMDP statistical tests produced by the Department of Biomathematics, University of California. The



version of BMDP used was converted for use on DEC 10/20 computers  
by the University of Pittsburg computer center.

## RESULTS

Behavioral acts made by rats were counted and averaged for all ten rats. The effects of alprazolam on the behavior of ten different isolated rats are shown in Table 2. Effects of triazolam on the behavior of ten different isolated rats are shown in Table 3. Control rats and rats treated by carboxymethyl cellulose also had their behaviors averaged and recorded in Table 1.

PCA-treated rats also had their behavioral acts counted and averaged for all ten rats. The effects of alprazolam on behaviors of ten rats are shown in Table 5, while the effects of triazolam on these behaviors are shown in Table 6. Control rats and rats treated by carboxymethyl cellulose for the drug-induced rats are averaged for all ten rats for each method and recorded in Table 4.

Aggressive acts of the control rats were compared to the aggressive acts of the seven treatments by a statistical t-test. Results were said to be significant at the 0.05 level. Only the t values of triazolam for the two lowest doses of the drug were found to have any significant effect on reducing total aggression in isolate rats. No significant effects were found for total submissive behaviors in rats that were isolated. Analysis of variance and covariance showed that there was no significant difference in which dose of the drug was used.

For rats that were treated by PCA, no significant changes were produced by either alprazolam or triazolam. Neither total aggressive or total submissive acts were changed significantly by

TABLE 1  
Control and Carboxymethyl Cellulose (CMC) Treated Rats  
for Isolation Treatment

	Control	CMC
Approach	4.1 $\pm$ 2.1	6.6 $\pm$ 2.2
Aggressive Posture	4.7 $\pm$ 3.0	5.5 $\pm$ 3.4
Aggressive Groom	4.6 $\pm$ 3.4	5.7 $\pm$ 3.3
Threat	5.4 $\pm$ 3.2	5.3 $\pm$ 2.5
Thrust	1.9 $\pm$ 2.2	1.8 $\pm$ 1.4
Attack	1.7 $\pm$ 2.3	1.8 $\pm$ 2.4
Offensive Upright	4.0 $\pm$ 2.6	2.9 $\pm$ 3.2
Offensive Sideways	3.2 $\pm$ 2.7	3.6 $\pm$ 4.9
Chase	0.6 $\pm$ 1.1	1.5 $\pm$ 2.1
Bite	0.7 $\pm$ 1.1	0.4 $\pm$ 1.2
Defensive Upright	2.0 $\pm$ 3.2	1.9 $\pm$ 4.3
Defensive Sideways	2.3 $\pm$ 1.6	1.2 $\pm$ 1.4
Submissive	0.7 $\pm$ 1.2	0.2 $\pm$ 0.6
Retreat	0.1 $\pm$ 0.3	0.3 $\pm$ 0.7
Flag	1.4 $\pm$ 1.3	2.7 $\pm$ 3.1
Evade	0.6 $\pm$ 0.7	0.2 $\pm$ 0.4
Crouch	0.7 $\pm$ 1.2	1.2 $\pm$ 1.9
Freeze	0	0.3 $\pm$ 0.5

n = 10

These are means and standard deviations.

TABLE 2  
Means and Standard Deviations for the Effects  
of Alprazolam on Isolated Rats

	Alprazolam (mg/kg)		
	0.5	1.0	2.0
Approach	5.9 $\pm$ 4.0	5.4 $\pm$ 4.0	6.0 $\pm$ 3.2
Aggressive Posture	4.7 $\pm$ 6.3	6.1 $\pm$ 8.9	6.1 $\pm$ 5.8
Aggressive Groom	2.4 $\pm$ 1.7	5.6 $\pm$ 7.6	1.9 $\pm$ 2.3 <sup>a</sup>
Threat	4.9 $\pm$ 5.0	3.5 $\pm$ 3.6	4.0 $\pm$ 4.7
Thrust	0.8 $\pm$ 0.9	1.0 $\pm$ 1.9	0.5 $\pm$ 0.7 <sup>a</sup>
Attack	1.8 $\pm$ 2.2	1.0 $\pm$ 1.8	0.8 $\pm$ 1.6
Offensive Upright	0.8 $\pm$ 1.4 <sup>c</sup>	1.3 $\pm$ 1.6 <sup>b</sup>	1.1 $\pm$ 1.5 <sup>b</sup>
Offensive Sideways	1.7 $\pm$ 2.5	1.4 $\pm$ 3.4	0.8 $\pm$ 1.7 <sup>a</sup>
Chase	0.9 $\pm$ 0.8	0.7 $\pm$ 1.3	0.5 $\pm$ 1.1
Bite	1.6 $\pm$ 3.6	7.7 $\pm$ 14.8	6.4 $\pm$ 8.5
Defensive Upright	1.4 $\pm$ 2.3	1.9 $\pm$ 4.0	0.6 $\pm$ 1.1
Defensive Sideways	0.6 $\pm$ 0.8 <sup>a</sup>	0 <sup>c</sup>	0.1 $\pm$ 0.3 <sup>c</sup>
Submissive	0.3 $\pm$ 0.7	0	0.1 $\pm$ 0.3
Retreat	0.2 $\pm$ 0.4	0	0.1 $\pm$ 0.3
Flag	2.5 $\pm$ 3.6	1.7 $\pm$ 1.7	1.0 $\pm$ 1.4
Evade	1.1 $\pm$ 1.7	0.2 $\pm$ 0.6	0.3 $\pm$ 0.6
Crouch	3.1 $\pm$ 4.1	6.4 $\pm$ 6.2 <sup>b</sup>	6.0 $\pm$ 4.4 <sup>c</sup>
Freeze	0.6 $\pm$ 1.1	1.2 $\pm$ 1.2	0.2 $\pm$ 0.4

n = 10

a = statistical significant at 0.05 level

b = statistical significant at 0.01 level

c = statistical significant at 0.005 level

TABLE 3

Means and Standard Deviations for the Effects  
of Triazolam on Isolation Induced Aggression

	Triazolam (mg/kg)		
	0.25	0.5	1.0
Approach	6.1 $\pm$ 3.4	5.1 $\pm$ 3.7	5.4 $\pm$ 2.1
Aggressive Posture	1.2 $\pm$ 1.7	3.8 $\pm$ 4.4	4.2 $\pm$ 4.0
Aggressive Groom	1.7 $\pm$ 2.0 <sup>a</sup>	3.2 $\pm$ 2.8	2.5 $\pm$ 1.7
Threat	2.9 $\pm$ 2.5	3.2 $\pm$ 1.8	3.1 $\pm$ 2.6
Thrust	0.3 $\pm$ 0.6 <sup>a</sup>	0.2 $\pm$ 0.6 <sup>a</sup>	0.4 $\pm$ 0.6 <sup>a</sup>
Attack	0.1 $\pm$ 0.3 <sup>a</sup>	0.1 $\pm$ 0.3 <sup>a</sup>	0.3 $\pm$ 0.6 <sup>a</sup>
Offensive Upright	0.2 $\pm$ 0.6 <sup>c</sup>	0.5 $\pm$ 0.7 <sup>c</sup>	0.6 $\pm$ 0.7 <sup>c</sup>
Offensive Sideways	0.2 $\pm$ 0.6	0.2 $\pm$ 0.6 <sup>c</sup>	1.4 $\pm$ 2.2 <sup>c</sup>
Chase	0.1 $\pm$ 0.3	0.1 $\pm$ 0.3	0.4 $\pm$ 0.5
Bite	1.4 $\pm$ 2.0	2.0 $\pm$ 2.1	3.6 $\pm$ 3.0 <sup>b</sup>
Defensive Upright	0.8 $\pm$ 0.9	1.3 $\pm$ 2.5	1.0 $\pm$ 1.5
Defensive Sideways	0 <sup>c</sup>	0.3 $\pm$ 0.6 <sup>c</sup>	0.2 $\pm$ 0.6 <sup>c</sup>
Submissive	0.1 $\pm$ 0.3	1.6 $\pm$ 3.1	1.1 $\pm$ 2.0
Retreat	0	0.1 $\pm$ 0.3	0.1 $\pm$ 0.3
Flag	0.7 $\pm$ 0.6	1.5 $\pm$ 1.1	2.1 $\pm$ 1.8
Evade	0.1 $\pm$ 0.3	0.1 $\pm$ 0.3	0.2 $\pm$ 0.4
Crouch	5.7 $\pm$ 4.5 <sup>c</sup>	7.8 $\pm$ 3.3 <sup>c</sup>	4.4 $\pm$ 3.5 <sup>c</sup>
Freeze	0	0.3 $\pm$ 0.7	0

n = 10

a = statistical significant at 0.05 level

b = statistical significant at 0.01 level

c = statistical significant at 0.005 level

TABLE 4

Control and Carboxymethyl Cellulose (CMC)  
Treated Rats for PCA Treatment Approach

	Control	CMC
Approach	4.5 $\pm$ 2.3	7.1 $\pm$ 3.3
Aggressive Posture	3.3 $\pm$ 2.4	5.3 $\pm$ 2.8
Aggressive Groom	8.7 $\pm$ 3.5	5.0 $\pm$ 3.0 <sup>c</sup>
Threat	4.4 $\pm$ 4.1	7.1 $\pm$ 4.0
Thrust	0.8 $\pm$ 1.3	1.3 $\pm$ 2.3
Attack	0.5 $\pm$ 1.1	2.2 $\pm$ 3.2
Offensive Upright	1.5 $\pm$ 2.2	4.0 $\pm$ 4.6
Offensive Sideways	2.2 $\pm$ 2.8	3.4 $\pm$ 6.4
Chase	0.7 $\pm$ 1.8	1.4 $\pm$ 3.0
Bite	2.7 $\pm$ 4.2	1.4 $\pm$ 2.1
Defensive Upright	0.6 $\pm$ 0.7	1.0 $\pm$ 1.9
Defensive Sideways	1.0 $\pm$ 1.1	0.8 $\pm$ 1.9
Submissive	1.0 $\pm$ 1.3	0.4 $\pm$ 0.5
Retreat	0.4 $\pm$ 1.3	0.3 $\pm$ 0.7
Flag	1.1 $\pm$ 1.3	3.3 $\pm$ 3.4
Evade	0.6 $\pm$ 1.3	0.4 $\pm$ 0.5
Crouch	1.9 $\pm$ 3.0	2.9 $\pm$ 3.2
Freeze	0.5 $\pm$ 0.9	0.2 $\pm$ 0.4

n = 10

c = statistical significant at 0.005 level

These are means and standard deviations.

TABLE 5  
Means and Standard Deviations for the Effects  
of Alprazolam on PCA-treated Rats

	Alprazolam (mg/kg)		
	0.5	1.0	2.0
Approach	4.5 $\pm$ 4.5	6.5 $\pm$ 5.1	5.1 $\pm$ 4.1
Aggressive Posture	6.5 $\pm$ 7.1	7.4 $\pm$ 7.0	4.2 $\pm$ 4.9
Aggressive Groom	1.6 $\pm$ 2.6 <sup>c</sup>	3.1 $\pm$ 3.7 <sup>c</sup>	4.0 $\pm$ 4.4 <sup>a</sup>
Threat	2.6 $\pm$ 2.2	3.4 $\pm$ 2.8	2.4 $\pm$ 3.5
Thrust	0.7 $\pm$ 1.1	1.1 $\pm$ 1.6	0.6 $\pm$ 1.0
Attack	0.7 $\pm$ 1.1	0.6 $\pm$ 1.3	0.8 $\pm$ 1.0
Offensive Upright	0.6 $\pm$ 1.1	1.0 $\pm$ 1.2	1.5 $\pm$ 3.1
Offensive Sideways	1.0 $\pm$ 1.2	1.3 $\pm$ 2.8	3.7 $\pm$ 5.6
Chase	0.3 $\pm$ 0.7	0.5 $\pm$ 0.8	0
Bite	4.3 $\pm$ 4.3	7.3 $\pm$ 9.2	3.4 $\pm$ 4.8
Defensive Upright	1.0 $\pm$ 1.3	1.0 $\pm$ 1.5	0.2 $\pm$ 0.4
Defensive Sideways	0.8 $\pm$ 1.1	0.2 $\pm$ 0.6	1.1 $\pm$ 2.8
Submissive	0.5 $\pm$ 0.9	0.5 $\pm$ 0.8	0.8 $\pm$ 1.4
Retreat	1.1 $\pm$ 2.8	0.9 $\pm$ 2.8	0.9 $\pm$ 2.5
Flag	1.8 $\pm$ 1.6	0.7 $\pm$ 1.1	0.5 $\pm$ 0.9
Evade	1.1 $\pm$ 1.7	0.3 $\pm$ 0.7	1.1 $\pm$ 1.9
Crouch	6.3 $\pm$ 5.8 <sup>a</sup>	7.0 $\pm$ 7.7	6.7 $\pm$ 8.1
Freeze	0.3 $\pm$ 0.7	0.1 $\pm$ 0.3	0.9 $\pm$ 2.2

n = 10

a = statistical significant at 0.05 level

c = statistical significant at 0.005 level

TABLE 6

Means and Standard Deviations for the Effects  
of Triazolam on PCA-treated Rats

	Triazolam (mg/kg)		
	0.25	0.5	1.0
Approach	4.8 $\pm$ 2.8	4.9 $\pm$ 2.2	5.9 $\pm$ 3.7
Aggressive Posture	5.8 $\pm$ 5.9	3.7 $\pm$ 2.5	5.8 $\pm$ 6.2
Aggressive Groom	2.9 $\pm$ 3.6 <sup>b</sup>	4.0 $\pm$ 3.3 <sup>b</sup>	6.4 $\pm$ 7.1
Threat	3.1 $\pm$ 2.0	1.7 $\pm$ 1.4	5.3 $\pm$ 5.0
Thrust	0.9 $\pm$ 0.9	0.2 $\pm$ 0.4	0.6 $\pm$ 1.8
Attack	0.1 $\pm$ 0.3	0.2 $\pm$ 0.4	0.1 $\pm$ 0.3
Offensive Upright	0.9 $\pm$ 1.1	0.7 $\pm$ 0.8	1.3 $\pm$ 2.1
Offensive Sideways	0.4 $\pm$ 0.6	1.1 $\pm$ 1.5	1.1 $\pm$ 2.4
Chase	0	0.1 $\pm$ 0.3	0
Bite	3.1 $\pm$ 4.7	3.6 $\pm$ 3.5	5.9 $\pm$ 5.4
Defensive Upright	1.2 $\pm$ 1.7	0	0.7 $\pm$ 1.3
Defensive Sideways	0.8 $\pm$ 1.9	0.2 $\pm$ 0.4	1.3 $\pm$ 1.8
Submissive	1.6 $\pm$ 2.1	0	1.0 $\pm$ 1.5
Retreat	0.7 $\pm$ 1.6	0	0.3 $\pm$ 0.5
Flag	3.0 $\pm$ 2.9	2.2 $\pm$ 2.4	2.9 $\pm$ 2.5
Evade	2.0 $\pm$ 3.2	0.5 $\pm$ 0.7	0.6 $\pm$ 1.1
Crouch	3.3 $\pm$ 3.1	3.9 $\pm$ 4.3	4.1 $\pm$ 4.1
Freeze	0	0.5 $\pm$ 0.8	0.7 $\pm$ 0.9

n = 10

b = statistical significant at 0.01 level

c = statistical significant at 0.005 level



by the two drugs.

Many of the individual behaviors had  $t$  values changed significantly at the 0.05 level, however. For the isolated rats, offensive upright was significantly reduced by both alprazolam and triazolam. Other isolate rat behaviors were also affected. Triazolam at a dose of 1.0 mg/kg increased bite behavior.

Crouch was increased at the 0.05 level by all three doses of triazolam, and the highest two doses of alprazolam. Offensive sideways was significantly reduced by the lowest two doses of triazolam and the highest dose of alprazolam.

Thrust was reduced by all doses of triazolam and the highest dose of alprazolam. Defensive sideways was found to be reduced at the 0.05 level by all doses of both drugs. Attack was reduced at the same level by the two lowest doses of triazolam and 2.0 mg/kg of alprazolam.

PCA-treated rats had much fewer significant changes in rat behavior. Crouch was increased by only 0.5 mg/kg of alprazolam. Aggressive groom was reduced by all three doses of alprazolam and the two lowest doses of triazolam. It was also significantly reduced by carboxymethyl cellulose.

Where all three doses of alprazolam or triazolam significantly changed behavior, analysis of variance and covariance was run to determine if increasing dose had any significant effect. Only two behaviors were found to be affected significantly. Increasing doses of alprazolam reduced defensive sideways behavior and increased crouch behavior in isolate rats.

## DISCUSSION

It is not surprising that total aggressive or submissive behaviors were only significantly affected by triazolam, which reduced aggressive behavior in isolated rats. Aggression as it occurs in animals and man is a series of behaviors, each of which could be affected differently by a particular drug. Since aggression in these experiments was measured as a series of individual behaviors, some of which were increased, and some that were decreased, it would be unusual if the combination of all these behaviors together yielded a significant result, unless all behaviors were affected the same way.

A majority of the work done with the effects of drugs on aggression in the past has been done with single response approaches. This previous research can only be compared with parts of this experiment, because of the multiple responses that were possible. All the behaviors used are important, however. Leavitt (1974) has found that changes in behavior other than fighting may be more important in determining aggressive behavior. According to Silverman (1965) these two methods of measuring aggression should be complementary, however.

It seems that without considering the underlying neural mechanisms of behavior, plus considering all the social responses that are possible, results cannot be very valid. All possible relevant behaviors for aggression and submission must be included.

In addition to these outward manifestations of drug effects, internal motivational effects of the test animal must also be considered. By considering all the behaviors separately in this experiment, and analysing them, the total effects of the drugs on aggression were shown.

Miczek and Barry (1976) found that it was often difficult to review the effects of drugs on aggression, because of its multiple causes. The separate behaviors often have different hormonal and neurological systems to regulate them, and each one of these systems can be affected differently by the drug used. In addition, aggressive acts may or may not result from another aggressive act, or possibly from a response of another animal.

When all these factors are taken into account, total aggression or total submission for these rats might not be affected by the two drugs used. Individual behaviors should be changed, however. It is difficult to say why triazolam decreased total aggression in isolated rats. It is possible that the individual behaviors that were decreased were lowered more than the behaviors that were increased were raised. This seems to be the case.

In comparison to the effects of alprazolam on isolated aggressive behavior, more behaviors were reduced by triazolam. It may be that triazolam reduces aggressive behavior better than alprazolam. Attack was only reduced by triazolam, while thrust and offensive sideways are reduced by more dosages of triazolam than of alprazolam. These particular behaviors are stronger in showing

aggressivity in the rats than some of the other behaviors, so it may be true that triazolam is stronger or more potent.

The way that the individual behaviors were affected by the two tranquilizers is interesting. Two behaviors were increased by the drug treatment, bite and crouch. While it was expected that crouch would be increased, the increase in bite is puzzling. It is possible that the increase in these two behaviors is related. The majority of the times crouch behavior was exhibited by the submissive rat, the other rat was performing an aggressive act. Most of the time this behavior was either bite or aggressive posture. Although both rats were not drugged, responses of the non-tranquilized rat had effects on the test animal. Both animals during the test exhibited both of these behaviors. One rat could be aggressive during one portion of the test, and submissive during another part of the test, and show both crouch and bite behavior.

As stated previously, the increase in crouch was expected. Drugs that have effects on cerebral serotonin have shown this effect in the past. Both tranquilizers affect serotonin in some manner. Ellison and Bresler (1974) found increases in two behaviors in rats during predatory threat situations when treated by parachlorophenylalanine. These two behaviors are the quiet motionless posture (freeze) and a rapid scurrying for cover. Freeze and crouch are fairly similar behaviors. It is surprising, however, that there was no increase in freeze or retreat behavior in this experiment.

Tenen (1967) found that reductions in serotonin decreased

spontaneous motor activity. This supports the theory that crouch would be increased. Also, a tranquilized animal should move less than a nondrugged animal. Both alprazolam and triazolam seem to be equally effective in increasing crouch in isolated rats. Only alprazolam seemed to be effective for drug-induced aggression, though. The reason less of increase in behavior was shown could be because crouch behavior was already higher in drug-induced control rats. This increase is probably due to the serotonergic effects already mentioned, since PCA lowers serotonin.

All of the remaining behaviors that were affected by the two tranquilizers were lowered from control levels. Behaviors that were lowered in isolated rats were strong aggressive behaviors for the most part. Exceptions to this are bite and aggressive groom. Bite is a fairly strong aggressive behavior that was increased, while aggressive groom is a low-level behavior that was lowered. Aggressive groom does bring the experimental animal into direct contact with the other animal, and this could be the reason it was reduced along with the strong aggressive behaviors.

Reduction in strong aggressive behaviors for isolate rats seems in accordance with the results found for other benzodiazepine tranquilizers. Although inconsistent results exist for the effects of benzodiazepines on aggression, the majority of data indicate a reduction in aggression and fighting behavior. Christmas and Maxwell (1970) found reduced aggression in mice and rats using four different benzodiazepines. Rudzik et al. (1973) have found reductions in aggression for shocked mice using four different

triazolobenzodiazepines, including alprazolam and triazolam.

Monkeys were also tamed by the two drugs.

This reduction of the strong aggressive elements is important. The strong aggressive behaviors would compare to fighting episodes or shock-induced bouts of aggression in other experiments. Other behaviors such as approach and threat may not be reduced because the stimulus needed to elicit these behaviors is lower. The two tranquilizers could raise the stimulus necessary for all behavior to take place and therefore only the weak behaviors could occur. This could also help explain why submissive behavior was largely unaffected.

DiMascio (1973) found increases in aggression at low doses, and reductions in aggression at high doses following administration of benzodiazepines. Greenblatt and Shader (1974) and Miczek (1974) have found similar results. The results of this thesis research did not seem to have this dose-dependent effect on aggression. Dosages of the two drugs either reduced aggression (except for bite) or had no effect on it.

This method of testing the effects of drugs on all aggressive and submissive behaviors has been used rarely in the past. Silverman (1965) used chlorpromazine, a major tranquilizer to reduce aggressive behavior in rats. No work has been done with minor tranquilizers using this method.

Silverman (1965) found all aggressive behaviors lowered, except for approach. Most submissive behaviors were increased except for defensive sideways, submission, and retreat. It was expected that

similar results would be found for this research. Since this did not happen, it is possible that high enough dosages of the two triazolobenzodiazepines were not used. Since major tranquilizers are much more potent, and less selective in their effects, it is possible that this is why the lesser aggressive behaviors were not affected for this thesis. At higher dosages, they might have been.

Results for drug-induced aggression show very little reduction in aggressive behavior by the two tranquilizers. Only aggressive groom was reduced by the two benzodiazepines in this method of producing aggression. Carboxymethyl cellulose also reduced this behavior, so it is possible that the control result was abnormally high, and there was no actual reduction by the tranquilizers. The reason there was a reduction in many aggressive behaviors for isolation-induced aggression but no reduction in drug-induced aggression is not known. Presumably, both these methods of inducing aggression affect serotonin in the same way, and similar results should be shown.

A possible explanation for the difference in the results could be a synergism between the effects of isolation and PCA. If this was the case, an increase in the drug-induced control behaviors should have been seen. This was not the case. It is possible, however, that aggressive responses are already as high as they can become in the isolation-induced control rats. Then any synergistic effect would have no bearing on aggression in the drug-induced control rats. Reduction of this by the two tranquilizers would only lower aggression to the high level found in isolated control

rats.

Another possible explanation for the difference in the results is that the rats were familiar to the method, or knew the other rats. Since the rats were placed together in a systematic manner, each with a different rat each time, this should not have had an effect. Plus, if this was the case, a great decrease in aggressive responses would have been expected, according to Miczek and Barry (1976). This did not happen, so these elements probably had no effect.

Triazolam is considered more potent than alprazolam. (Rudzik et al., 1973). This may be important, although lower dosages of triazolam were used in this experiment to try and compensate for this effect. It is interesting that only the highest dose of alprazolam was effective in reducing aggression for thrust and offensive sideways in isolated rats. All three doses of triazolam reduce thrust, while the two lowest doses of triazolam reduce offensive sideways.

It is doubtful from data that dosage was important for the effects of triazolam. Many of the individual behaviors were changed by the two lowest dosages of triazolam, but not the highest dose, while bite was increased only by the highest dosage. It was thought that increasing dosage would bring about a greater reduction in aggression. This seems not to be the case for this drug. Higher dosages seem to lose their effectiveness. More doses would be needed to test this theory, however. Since the same rats were used for all doses, some familiarity to the experimental



process may have existed, or residual effects from the other dose may have existed. This could explain the reduction in effectiveness at higher dosages. This is doubtful, however, when the results for alprazolam are considered.

Dosage does seem to be important for the effects of alprazolam. For many of the individual behaviors, only the highest dosage, or the two highest dosages were effective in reducing aggression. In addition, increasing dosage of alprazolam reduced defensive sideways and increased crouch behavior in isolated rats. It would seem that if familiarity did develop to the effects of triazolam, alprazolam would be affected the same way. This did not happen.

In drug-induced aggression, alprazolam seems to be more effective for what little changes in behavioral elements did occur. Which of the two drugs was more effective in reducing isolation-induced aggressive behavior is questionable. As stated before, triazolam seemed to reduce more individual behaviors than alprazolam. Except that it is more potent, triazolam seems to work no better than alprazolam in reducing the behaviors that they both reduce.

### Summary and Conclusions

It seems that benzodiazepine tranquilizers calm many aggressive behaviors in isolated rats. Rats made aggressive by drugs seem unaffected by the two tranquilizers, however. It is not known why the drug-induced rats were not affected. Both alprazolam and

triazolam seem to reduce some aggressive behaviors in isolated rats, but triazolam is stronger. The effects are also analogous to the effects of other benzodiazepines, such as chlordiazepoxide and diazepam. However, alprazolam and triazolam are more potent than diazepam, and much more potent than chlordiazepoxide. The strength of these drugs could be an important factor in some experiments, and in use by humans.

All methods and drugs used in this experiment had the effect of reducing serotonin in the brain. This seems contradictory, however. If isolation of rats, and drugs such as PCA decrease serotonin, and produce aggression, how is it possible that benzodiazepines which reduce aggression also inhibit serotonin. Unless serotonin has no bearing on the effects of benzodiazepines, or has no causal effect on aggression, and this seems unlikely from previous research, this factor needs to be investigated further. Other central nervous system depressants raise brain 5-HT (Anderson and Bonnycastle, 1960). More evidence is needed to clear up the effects of benzodiazepines on serotonin, and how brain amines affect aggression.

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