

Effects of Alprazolam and Triazolam on Isolation-Induced Aggression in Rats^{1,2}

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ABSTRACT. The efficacy of two new benzodiazepine tranquilizers, alprazolam and triazolam, in the reduction of aggression produced in rats by isolation was tested. Rats were isolated for at least three weeks. They were then placed in a cage with another rat, and aggressive and submissive behaviors that occurred were noted. Several of the behaviors were changed by either alprazolam or triazolam. Aggressive behaviors of thrust, attack, offensive upright, and offensive sideways were decreased; bite was increased. The submissive behavior of defensive sideways was decreased, whereas crouch and freeze were increased. Reduction in aggression seems consistent with results for other benzodiazepine tranquilizers such as diazepam and chlordiazepoxide.

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INTRODUCTION

Studies of the influence of psychotropically active benzodiazepines on reduction of aggression have provided conflicting results. Chlordiazepoxide, diazepam, and other benzodiazepine tranquilizers have variable effects on aggressive behaviors in animals depending on species, strain, sex, social environment, and method used to induce aggressive behavior (Greenblatt and Shader 1974, DiMascio 1973, Miczek 1974, Valzelli 1973, Malick et al. 1969, Dantler 1977, Cook and Kelleher 1963, Christmas and Maxwell 1970, Olivier and Van Dalen 1982).

Little work has been done with two new benzodiazepines, alprazolam and triazolam, which are triazolobenzodiazepines. Triazolobenzodiazepines differ from diazepam by addition of a five-membered ring at position one and two of the benzodiazepine structure, which increases the potency.

Previous studies on rodents indicated that alprazolam and triazolam affect aggression. For example, Ueki et al. (1981) found that alprazolam suppressed aggression and muricide by olfactory-bulbectomized rats. Taming effects on aggressive mice and monkeys were found by Castaner and Chatterjee (1976). Triazolam also reduced aggression produced by septal lesions or by olfactory-bulbectomized rats (Ueki et al. 1978). Gomito et al. (1978) found that conditioned behavior in rats that were in a conflict situation induced by footshock was reduced by triazolam. Both drugs reduce shock-induced aggression in mice (Rudzik et al. 1973).

This study was designed to show the effects of alprazolam and triazolam on isolation-induced aggression in rats. The isolation method (Yen et al. 1959) was used because it produced aggression that resembles naturally occurring aggression in both rodents and man.

The effects of the two tranquilizers on aggressive and submissive behaviors of rats (Grant and Mackintosh 1963) were observed. It was hypothesized that aggressive behaviors would be decreased, and that submissive behaviors would be increased in rats that were tranquilized. As previously stated, many investigators reported results for only single behavioral events in the past. By testing more behavioral parameters, a more complete response to

the drugs for the various aggressive and submissive behaviors was found.

MATERIALS AND METHODS

Male albino rats were acquired from the R. G. Sewell Laboratory in the Behavioral Effects of Cancer Therapy, Department of Psychology, Western Michigan University. To study aggression, the rats were isolated from one another visually for at least three weeks. They were housed singly in cages 18 × 23 × 18 cm. The cages, which had solid sides and backs, with wire fronts and bottoms, were placed in a rack in two rows of five cages each. Each cage was separated from the other cages.

All rats weighed from 400 to 550 g, and were provided with Purina rat chow and water *ad libitum*. The colony room was illuminated continuously and was maintained at a constant temperature of 21°C. Ten rats were used for each of the four phases of the study.

The first phase of the experiment was the control phase. After the 3-week period of isolation, each rat was placed in an experimental test chamber with another rat for 30 min. Each behavior listed below was recorded each time that it occurred during the 30-min trial. After the test, the rats were returned to their respective cages.

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

- 1) **Aggression**
 - a) Approach—a movement toward the other rat.
 - b) Aggressive posture—the aggressive animal orients itself at right angles to and over the other rat.
 - c) Aggressive groom—nibbling or grooming fur of the other rat.
 - d) Threat—head movement toward the other rat.
 - e) Thrust—movement of the whole forefront of the aggressive rat's body toward the other rat.
 - f) Attack—rapid movement toward the other rat.
 - g) Offensive upright—aggressive rat stands on hind legs, head oriented toward the other rat.
 - h) Offensive sideways—aggressive rat approaches the other rat broadsides.
 - i) Chase—chasing the other rat.
 - j) Bite—biting the other rat.
- 2) **Flight, Escape and Submission**
 - a) Defensive upright—rat on hind legs, but his head is not oriented toward the other rat.
 - b) Defensive sideways—rat presents its side to the other rat.
 - c) Submissive—rat lies on its back.
 - d) Retreat—movement away from the other rat.
 - e) Flag—movement of head away from the other rat.
 - f) Evade—movement of the whole forefront of the body away from the other rat.
 - g) Crouch—rat is on all four paws, often has its shoulders lowered.
 - h) Freeze—rat does not move.

Before injection both tranquilizers were dissolved in 0.5% carboxymethyl cellulose (CMC). The second phase of the experiment involved testing the effects of CMC on a second set of 10 rats. These rats were tested in exactly the same ways as the untreated rats. A third set of 10 rats was tested with alprazolam at doses of 0.5, 1.0, and 2.0 mg/kg; a fourth set of 10 rats was tested with triazolam at doses of 0.25, 0.5, and 1.0 mg/kg. Because triazolam is considered to be more potent than alprazolam (Rudzik et al., 1971), lower doses of triazolam were used in the experiment to compensate for this.

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In phases 2, 3 and 4 the rats were injected 20 min. prior to the start of the test and placed in the experimental chamber. This was done to produce the peak effect of the drug during the test (Kitagawa et al. 1979). Rats were kept in two separate rows of five cages each. An experimental rat from one row was placed with a rat from another row; no two rats were placed together more than once.

Since the majority of the data derived from the experiments were not normally distributed, nonparametric statistics were used for analysis. Data from untreated rats and CMC-treated rats were compared by a Mann-Whitney *U*-test. Since only one behavior was affected by carboxymethyl cellulose, the results of the two were combined and compared with a Kruskal-Wallis test to rats receiving alprazolam and triazolam (Sokal and Rohlf 1969). Since the data were non-normal, a dose-response analysis was not done.

RESULTS

The results of the experiments are shown in Table 1. Control rats were compared to rats treated with carboxymethyl cellulose. It was found that the only behavior that changed significantly ($P < 0.05$) was approach, which increased.

Data from control rats and the experimental rats were compared by the Kruskal-Wallis test. The aggressive behaviors that decreased were thrust ($P < 0.001$, Fig. 1), attack ($P < 0.01$, Fig. 2), offensive upright ($P < 0.001$, Fig. 3), and offensive sideways ($P < 0.01$, Fig. 4). The only aggressive behavior that increased was bite ($P < 0.01$, Fig. 5).

The submissive behavior that was decreased was defensive sideways ($P < 0.001$, Fig. 6). Submissive behaviors that increased were crouch ($P < 0.001$, Fig. 7), and freeze ($P < 0.01$, Fig. 8).

DISCUSSION

It was hypothesized that aggression would decrease in the tranquilized rats, and submissive behaviors would increase. Two submissive behaviors, crouch and freeze, were increased by the drug treatment. This increase could

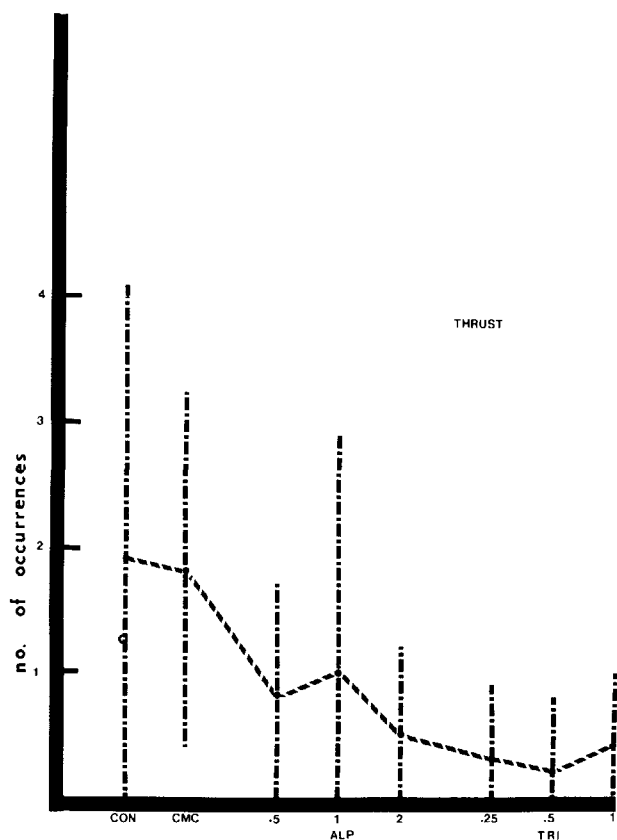


FIGURE 1. Effects of alprazolam and triazolam on number of occurrences of thrust behavior over a 30-min period. Doses of the two drugs are given in mg/kg. Vertical dashed lines represent standard deviations. CON, control; ALP, alprazolam; TRI, triazolam.

TABLE 1.
Means and standard deviations of effects of alprazolam and triazolam on isolation-induced aggression in rats.

Behavior	Control	Carboxymethyl cellulose	Alprazolam (mg/kg)			Triazolam (mg/kg)		
			0.5	1.0	2.0	0.25	0.5	1.0
Approach	4.1 ± 2.1	6.6 ± 2.2	5.9 ± 4.0	5.4 ± 4.0	6.0 ± 3.2	6.1 ± 3.4	5.1 ± 3.7	5.4 ± 2.1
Aggressive posture	4.7 ± 3.0	5.5 ± 3.4	4.7 ± 6.3	6.1 ± 8.9	6.1 ± 5.8	1.2 ± 1.7	3.8 ± 4.4	4.2 ± 4.0
Aggressive groom	4.6 ± 3.4	5.7 ± 3.3	2.4 ± 1.7	5.6 ± 7.6	1.9 ± 2.3	1.7 ± 2.0	3.2 ± 2.8	2.5 ± 1.7
Threat	5.4 ± 3.2	5.3 ± 2.5	4.9 ± 5.0	3.5 ± 3.6	4.0 ± 4.7	2.9 ± 2.5	3.2 ± 1.8	3.1 ± 2.6
Thrust*	1.9 ± 2.2	1.8 ± 1.4	0.8 ± 0.9	1.0 ± 1.9	0.5 ± 0.7	0.3 ± 0.6	0.2 ± 0.6	0.4 ± 0.6
Attack**	1.7 ± 2.3	1.8 ± 2.4	1.8 ± 2.2	1.0 ± 1.8	0.8 ± 1.6	0.1 ± 0.3	0.1 ± 0.3	0.3 ± 0.6
Offensive upright*	4.0 ± 2.6	2.9 ± 3.2	0.8 ± 1.4	1.3 ± 1.6	1.1 ± 1.5	0.2 ± 0.6	0.5 ± 0.7	0.6 ± 0.7
Offensive sideways**	3.2 ± 2.7	3.6 ± 4.9	1.7 ± 2.5	1.4 ± 3.4	0.8 ± 1.7	0.2 ± 0.6	0.2 ± 0.6	1.4 ± 2.2
Chase	0.6 ± 1.1	1.5 ± 2.1	0.9 ± 0.8	0.7 ± 1.3	0.5 ± 1.1	0.1 ± 0.3	0.1 ± 0.3	0.4 ± 0.5
Bite***	0.7 ± 1.1	0.4 ± 1.2	1.6 ± 3.6	7.7 ± 14.8	6.4 ± 8.5	1.4 ± 2.0	2.0 ± 2.1	3.6 ± 3.0
Defensive upright	2.0 ± 3.2	1.9 ± 4.3	1.4 ± 2.3	1.9 ± 4.0	0.6 ± 1.1	0.8 ± 0.9	1.3 ± 2.5	1.0 ± 1.5
Defensive sideways*	2.3 ± 1.6	1.2 ± 1.4	0.6 ± 0.8	0	0.1 ± 0.3	0	0.3 ± 0.6	0.2 ± 0.6
Submissive	0.7 ± 1.2	0.2 ± 0.6	0.3 ± 0.7	0	0.1 ± 0.3	0.1 ± 0.3	1.6 ± 3.1	1.1 ± 2.0
Retreat	0.1 ± 0.3	0.3 ± 0.7	0.2 ± 0.4	0	0.1 ± 0.3	0	0.1 ± 0.3	0.1 ± 0.3
Flag	1.4 ± 1.3	2.7 ± 3.1	2.5 ± 3.6	1.7 ± 1.7	1.0 ± 1.4	0.7 ± 0.6	1.5 ± 1.1	2.1 ± 1.8
Evade	0.6 ± 0.7	0.2 ± 0.4	1.1 ± 1.7	0.2 ± 0.6	0.3 ± 0.6	0.1 ± 0.3	0.1 ± 0.3	0.2 ± 0.4
Crouch***	0.7 ± 1.2	1.2 ± 1.9	3.1 ± 4.1	6.4 ± 6.2	6.0 ± 4.4	5.7 ± 4.5	7.8 ± 3.3	4.4 ± 3.5
Freeze****	0	0.3 ± 0.5	0.6 ± 1.1	1.2 ± 1.2	0.2 ± 0.4	0	0.3 ± 0.7	0

* Behavior decreased from control level ($P < 0.001$).

** Behavior decreased from control level ($P < 0.01$).

*** Behavior increased from control level ($P < 0.001$).

**** Behavior increased from control level ($P < 0.01$).

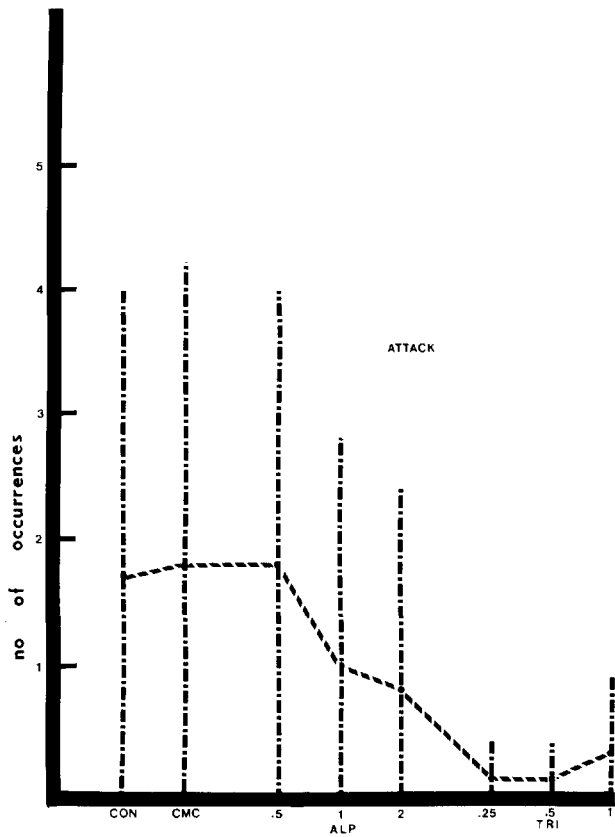


FIGURE 2. Effects of alprazolam and triazolam on attack behavior over a 30-min period. Details as in Fig. 1.

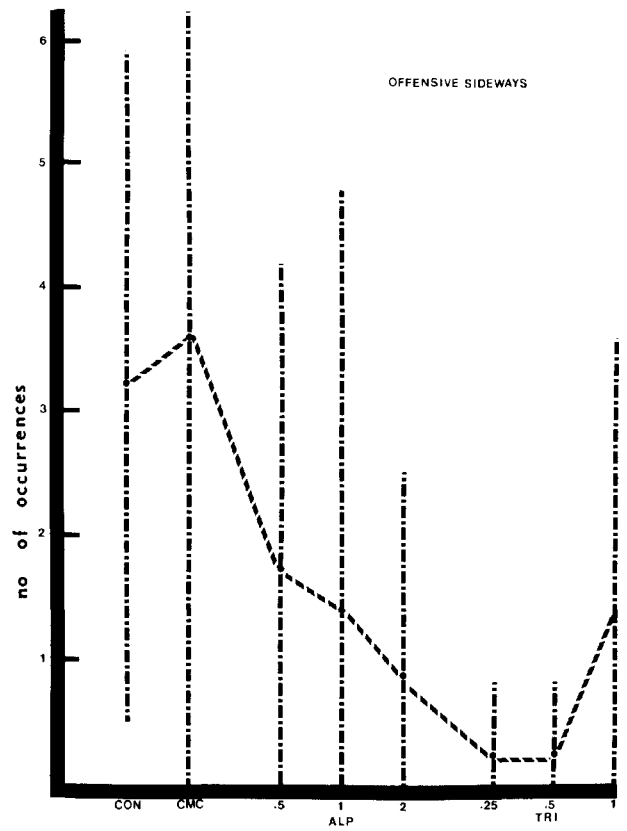


FIGURE 4. Effects of alprazolam and triazolam on offensive sideways behavior over a 30-min period. Details as in Fig. 1.

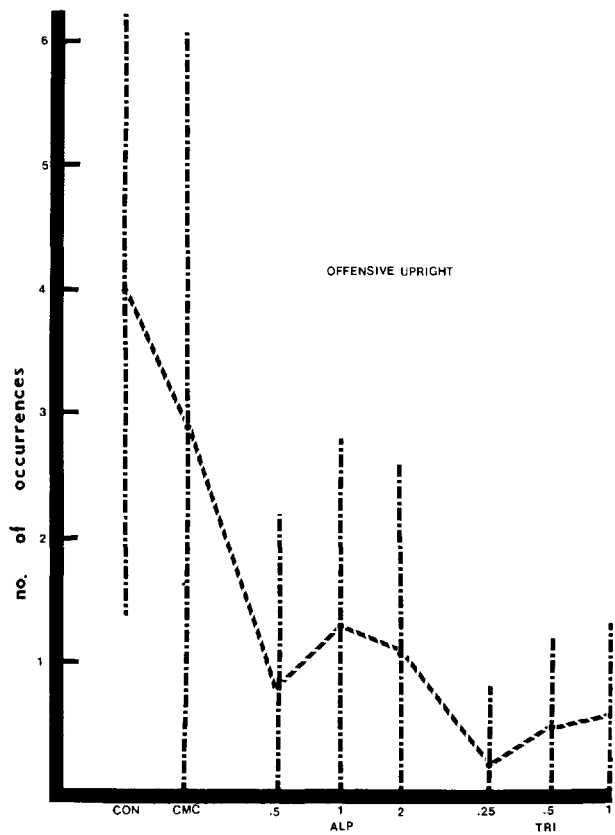


FIGURE 3. Effects of alprazolam and triazolam on offensive upright behavior over a 30-min period. Details as in Fig. 1.

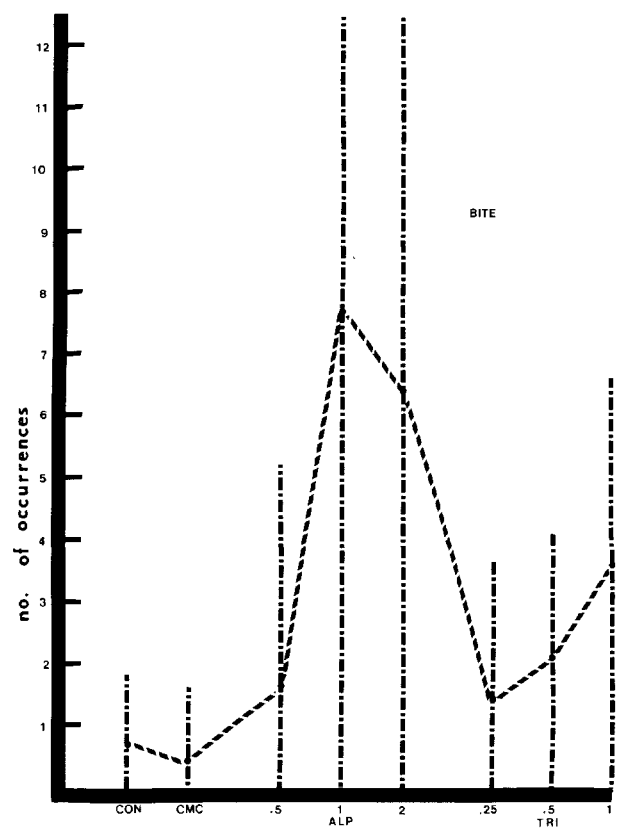


FIGURE 5. Effects of alprazolam and triazolam on bite behavior over a 30-min period. Details as in Fig. 1.

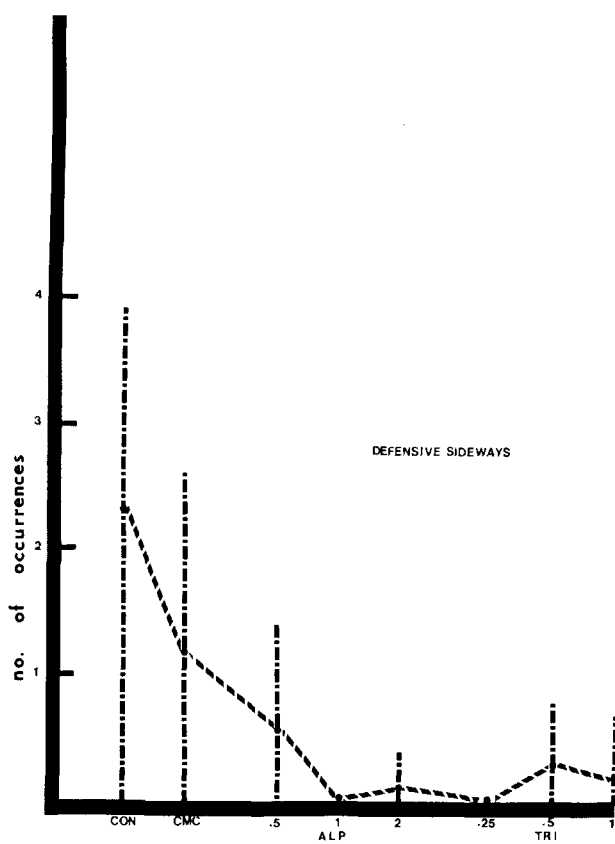


FIGURE 6. Effects of alprazolam and triazolam on defensive sideways behavior over a 30-min period. Details as in Fig. 1.

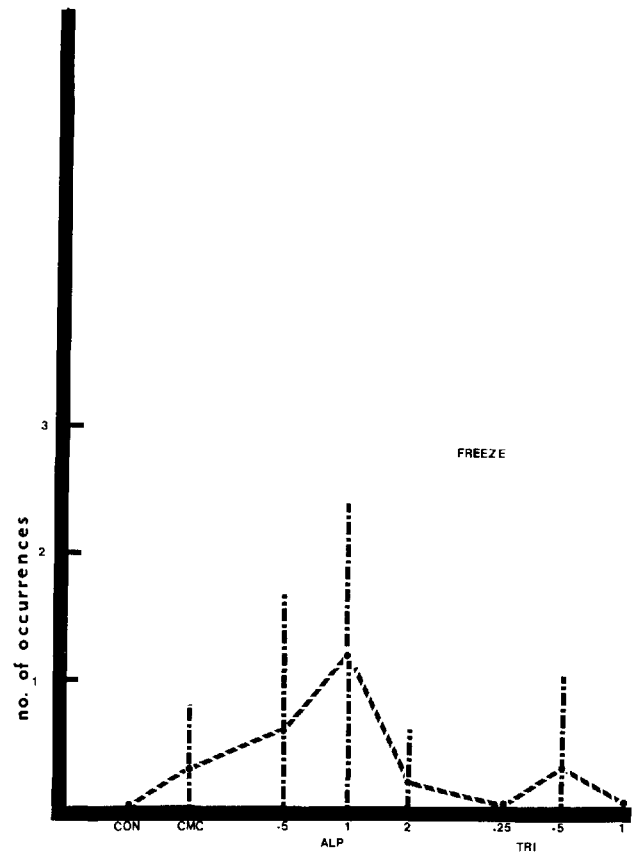


FIGURE 8. Effects of alprazolam and triazolam on freeze behavior over a 30-min period. Details as in Fig. 1.

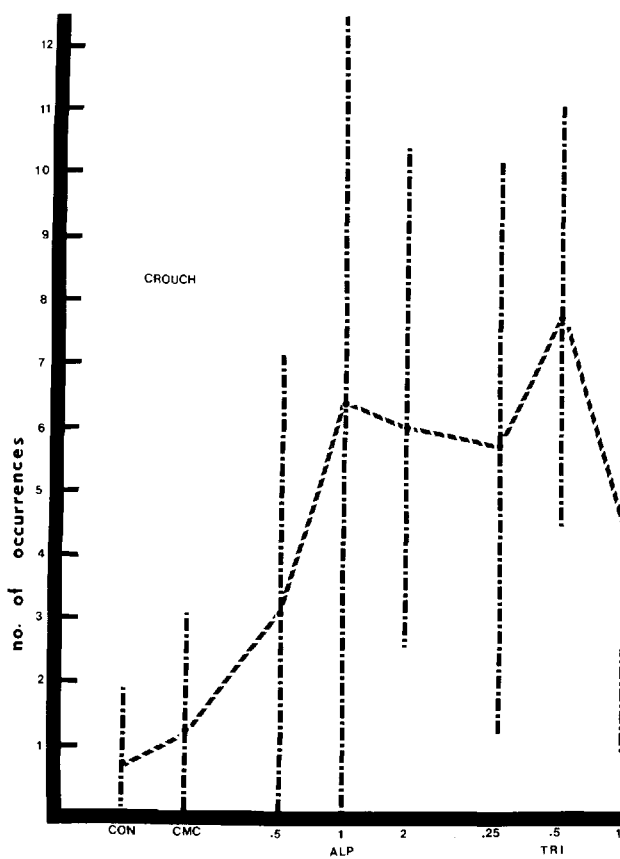


FIGURE 7. Effects of alprazolam and triazolam on crouch behavior over a 30-min period. Details as in Fig. 1.

be related to the increase in bite behavior. The majority of times that crouch was exhibited by the submissive rat, the other rat was performing an aggressive act, usually bite or aggressive posture. The increase in crouch and freeze was expected. File (1981) reported that one of the effects of benzodiazepines is to decrease spontaneous locomotor activity. It was surprising, however, that no other submissive behaviors were increased in the present study.

Strong aggressive behaviors such as threat, attack, offensive upright, and offensive sideways were decreased for the most part by the two tranquilizers, but not significantly for both as expected. Although inconsistent results exist for the effects of benzodiazepines on aggression and fighting behavior. DiMascio (1973) found that isolation-induced aggression was reduced only at high dose levels of several benzodiazepines, principally diazepam, chlordiazepoxide, and nitrazepam. Valzelli (1973) observed that isolation-induced aggression decreased in rats and mice dosed with several different benzodiazepines. Malick (1978) noted that fighting behavior in mice was reduced by diazepam following chronic administration of the drug. Krsiak (1974) reported that singly housed mice showed more aggressive postures at low doses. Cook and Kelleher (1963) found that this type of aggression was unaffected by benzodiazepines, whereas Fox et al. (1970) noted that aggression increased in male mice treated with chlordiazepoxide when grouped together. Olivier and Van Dalen (1982) reported that aggression increased in mice and rats treated with chlordiazepoxide.

The reduction in the strong aggressive elements is important with respect to the studies mentioned above. The strong aggressive elements would compare to fighting episodes in these experiments, or shock-induced bouts of aggression in other experiments. Other behaviors such as approach and aggressive posture may not be reduced for other reasons. It is expected that these more general behaviors would be affected more by the tranquilizers. This was not the case in the present study. It is also possible that the strong aggressive elements and these more general behaviors have different mechanisms of action, and that the benzodiazepines only affect the strongly aggressive behaviors.

This method of testing the effects of drugs on all aggressive and submissive behaviors has been rarely used in the past. Silverman (1965) used chlorpromazine to reduce aggressive behavior in rats, and found that all aggressive behaviors were reduced except for approach. Most submissive behaviors were increased, except for defensive sideways, submission and retreat. It was expected that similar results would be found in the present study. Since they were not observed, it is possible that insufficient doses of the two triazolobenzodiazepines were used. Since major tranquilizers such as chlorpromazine are much more potent and less selective in their effects than benzodiazepines, it is possible that this is the reason why the less aggressive behaviors were not affected. Benzodiazepines could be more selective in their actions on these behaviors. Higher doses of the drugs could be used in further research, however. Olivier and Van Dalen (1982) found that aggression and social behavior increased when they used this method of testing all behaviors.

Many benzodiazepines mediate their activity by binding to sites within the rat brain (Braestrup and Squires 1978, Lippa et al. 1978). Squires and Braestrup (1977) found that these sites are distributed unevenly throughout the rat brain, and that they correlate with their anxiolytic effects. Ongini et al. (1982) reported similar results for mice. Sethy and Harris (1982) found that alprazolam and triazolam mediate their behavioral effects by binding to receptors in the brain. It is not known if these receptors affect only the behaviors (i.e. strongly aggressive behaviors) altered in this experiment and not more generally social behaviors such as approach. This is a possible hypothesis since these receptors are known to reduce anxious behavior, and since more general behaviors are probably not anxiolytic.

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