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β-Carboline FG 7142-Reduced Aggression in Male Rats: Reversed by the Benzodiazepine Receptor Antagonist, Ro15-1788[1]

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BECK, C. H. M. AND S. J. COOPER. *β-Carboline FG 7142-reduced aggression in male rats: Reversed by the benzodiazepine receptor antagonist, Ro15-1788.* PHARMACOL BIOCHEM BEHAV 24(6) 1645–1649, 1986.—The β-carboline FG 7142 decreases conspecific aggression in male hooded rats. The purpose of this study was to examine the effects of pretreatment with Ro15-1788 or chlordiazepoxide (CDP) in this paradigm. The six groups (n=8) were saline, FG 7142 (5 mg/kg, immediate, IP), CDP (5 mg/kg, −10 min, IP), CDP (5 mg/kg, −10 min) plus FG 7142 (5 mg/kg, immediate), Ro15-1788 (10 mg/kg, −10 min, IP), and Ro15-1788 (10 mg/kg, −10 min) plus FG 7142 (5 mg/kg, immediate). Following injection of the more aggressive member of a pair of isolation-housed rats, the pair was observed in a living cage over four 6-min trials interpolated over a 40 min session. In the first 20 min after the injection FG 7142 decreased aggression, decreased the pinning of the other animal, and increased avoiding behavior. These effects were the opposite of those seen in the Ro15-1788-injected rats and Ro15-1788 pretreatment reversed the effects of FG 7142. CDP alone caused prolonged aggressive behavior but as a pretreatment only partially reversed the effects of FG 7142.

β-Carboline Benzodiazepine FG 7142 Ro15-1788 Aggression Social behavior Ethological analysis Rat

FG 7142 is a partial inverse agonist of benzodiazepine receptors. Treatment with FG 7142 reduces active social contact between male rats in a social interaction test [6]. Little aggression is observed between animals in this test, their contacts being mostly investigative or affiliative. Tests involving conspecific aggression provide evidence that FG 7142 treatment reduces aggression [1,8]. Whereas aggressive behavior increased and avoiding behavior decreased, total-social behavior was not changed in FG 7142-treated rats relative to control animals [1]. One goal of the present study was to determine firstly if this FG 7142 specificity for agonistic behavior could be confirmed and secondly whether it would be robust enough to appear in animals housed in isolation. Drug effects observed in animals housed in pairs [1] may not be generalizable to those obtained from animals housed individually because the normal social behavior of rats tested in pairs is strongly influenced by whether the animals are housed in isolation or in groups [12–13, 18].

The primary goal of the study was to determine the involvement of the benzodiazepine receptor in the FG 7142 effect by pretreating the animals with the specific benzodiazepine receptor antagonist Ro15-1788 [9]. We also examined the consequences of acute pretreatment with the benzodiazepine agonist, chlordiazepoxide, for the FG 7142 effect. Ancillary goals of this portion of the study were to describe the effects of chlordiazepoxide treatment alone with those of Ro15-1788 alone. In a study in which only one drug treatment was administered per rat, CDP had no acute effect on the animal's aggressive behavior [5]. Studies involving more than one treatment per rat found that CDP acutely produced an increase [15] or a decrease in aggression [19]. The effect of Ro15-1788 on aggressive behavior in rats has not been reported.

METHOD

Animals

Ninety-six male hooded rats (General strain, Department of Psychology, University of Birmingham) were housed individually beginning two weeks prior to the time of testing. The animal quarters were maintained at 22± 1°C. Lights were on between 08.30 and 20.30 hr. Food and water were freely available. The rats weighed 230–310 g at the start of testing.

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Apparatus

The animals were tested in a metal living cage measuring 27×24×21 cm high. The grid floor, which was marked off into quadrants, covered a drop pan filled with litter. The test cage was different from the standard living cage in that the metal ceiling was replaced with a transparent sheet of plastic and the food and water dispensers were empty. The test cage was located inside a grey wooden box 55×55×50 cm high. A 15 watt red light bulb hung 6 cm above the cage. A mirror was suspended at 45° above the top of the box to permit the observer to view the animals from above.

Drug Treatments

FG 7142 (Ferrosan) and Ro15-1788 (Hoffmann-La Roche) were suspended by ultrasonic dispersion in distilled water with one drop of Tween 80 per ml. Chlordiazepoxide HCl (Hoffmann-La Roche) was dissolved in saline immediately before use. All injections were delivered IP in a volume of 1 ml/kg. Doses are expressed as weight of the salt.

Procedure

Weight-matched pairs of rats were adapted to handling and scored in three homecage sessions for the number of pins and supines [15]. The more aggressive member of the pair was chosen as the experimental animal to be injected and observed in the experimental sessions. Following the handling sessions, one further session was held in which each rat was injected IP with saline and adapted individually to the test cage in the test room for 20 min.

At the start of formal testing the 48 pairs of rats were randomly allocated, 8 pairs to each of 6 treatments: controls (distilled water and Tween, 1 ml/kg, −10 min plus 0.9% NaCl, 1 ml/kg, immediate), FG 7142 (5 mg/kg, immediate), chlordiazepoxide (5 mg/kg, −10 min), chlordiazepoxide plus FG 7142 (CDP 5 mg/kg, −10 min plus FG 7142 5 mg/kg, immediate), Ro15-1788 (10 mg/kg, −10 min), Ro15-1788 plus FG 7142 (Ro15-1788 10 mg/kg, −10 min plus FG 7142 5 mg/kg, immediate). These groups are subsequently referred to as controls, FG, CDP, CDP-FG, Ro, and Ro-FG respectively. The CDP dose used has been reported to be effective in reversing the effects of FG 7142 on social behavior [6]. The order of testing of the treatment pairs within test days was randomized. Testing was done between 08.30 and 14.00 hr.

The experimental session consisted of four 6-min observation trials with an intertrial interval of 4 min. The first trial began after 2-min of adaptation to the test cage. The observer recorded the experimental rat's behavior continuously throughout each observation trial by entering coded symbols for thirteen comprehensive and exclusive behavioral categories on a microprocessor key board. The microprocessor was programmed to store the behavioral codes and their time of entry. The behavior categories were *locomote-environment*, the rat's forequarters moving into a quadrant of the cage, the bodily movement being other than approaching, avoiding or attacking the other rat; *sniff-environment*, continuous small scanning movements of the head while examining the environs; *inactive-environment*, sitting or lying with eyes open without bodily movement, while not in bodily contact with the other rat excluding instances of freezing after being attacked; *groom*, grooming, sniffing, washing, combing, licking, or scratching itself; *approach*, investigating the other rat by sniffing or grooming, moving over or under the other rat, all of this in a manner evoking either mutual investigation or no response from the recipient; *locomote-approach*, the moving of the rat's forequarters into a cage quadrant pursuant to approach; *avoid*, head and body movements during or after an attack, being prone, supine, or rearing; *locomote-avoid*, moving of the rat's forequarters into a cage quadrant when avoiding attack; *aggress*, attacking, biting, kicking showing lateral threats, circling with arched back and limbs extended, and arching over partner and grooming back [15] and in addition any active contact with the other rat evoking locomote-avoid, avoid, locomote-aggress, or aggress; *locomote-aggress*, moving of the rat's forequarters into a cage quadrant when engaged in aggress; *inactive-social*, identical to inactive-environment except that the rat is in passive bodily contact with the other rat; *sleep*, sleeping as indicated by closed eyes, curled tail and head at rest. In addition a record was kept of the frequency of pins, supines, and seizures.

Nine composite behavior categories were derived by grouping selected behavior categories. The composite behaviors were *nonsocial* behavior, including locomote-environment, sniff-environment, inactive-environment, and groom; *investigative* behavior, including approach and locomote-approach; *avoiding* behavior, including avoid and locomote-avoid; *aggressive* behavior, including aggress and locomote-agress; *passive-social* behavior, including inactive-social and sleep because sleep was always done in contact with the other rat; *active-social* behavior, including investigative, avoiding, and aggressive behaviors; *total-social* behavior, including active and passive social behaviors; *locomotion*, including all four locomote behaviors; and *immobility*, including inactive-social, inactive-environment, avoid, and sleep. Avoid was included in immobility because for most of the FG 7142-injected animals avoiding behavior consisted of crouching immobile.

The three measures of the behavioral categories and their composites were *bout frequency*, the number of nonconsecutive occurrences, i.e., bouts, of a behavior per observation trial; *bout duration*, the mean duration(s) of the bouts of a behavior per trial; *percent time*, the percent of total time in a trial spent excuting a behavior. Test-retest correlations of scoring of video-taped reruns of drugged and control rats indicated the observer's coding reliability to be significant with agreements at 80% or better. Interjudge reliability based on comparisons of the scores of the observer and those of an independent observer produced agreements in the 76 to 97% range. All correlations were significant $p<0.05$.

Separate two-way ANOVAs of groups by trials were computed for each dependent measure on each behavior category. The three measures were per trial bout frequency scores (BF), percent time scores (PT), and transformed mean bout duration scores (BD), wherein X in seconds = square root of X + 1. Tukey tests were used to assess group differences within trials and trial differences within groups. Statements of Tukey test significance reported in the results imply $p<0.05$.

RESULTS

The data on particular behaviors are not presented because of the following factors, low incidence (inactive-environment, inactive-social, supine, sleep, and passive social) redundancy with other behaviors (immobility, active-social, and nonsocial), and absence of significant effects (supine). The composite categories, investigative, avoiding,

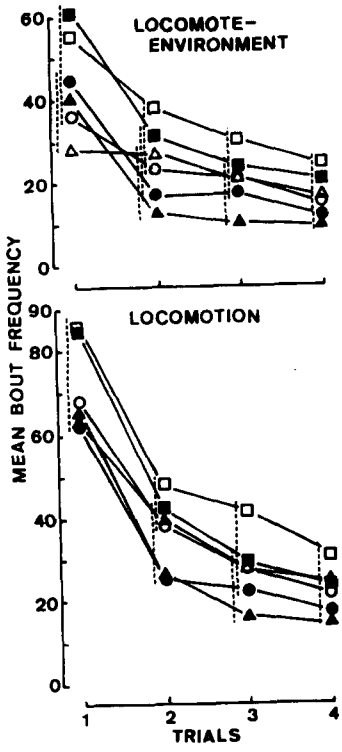

FIG. 1. Mean bout frequency for locomote-environment and locomotion over 4 trials for 6 groups (n=8) saline (open circles), FG 7142 (filled circles), CDP (open triangles), CDP plus FG 7142 (filled triangles), Ro15-1788 (open squares), and Ro15-1788 plus FG 7142 (filled squares). Group means which are not linked by the dotted line on the left are significantly different (Tukey, $p<0.05$). Group means which are linked by the dotted line are not significantly different. S.E.s are less than 15% of mean values.

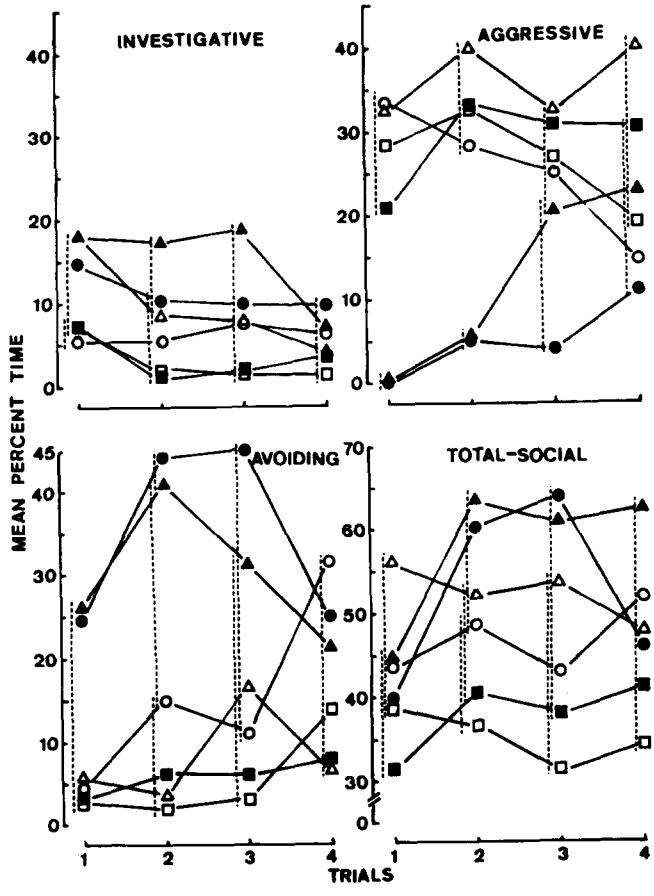

FIG. 2. Mean percent time for investigative, aggressive, avoiding and total-social behavior over 4 trials for 6 groups (n=8) saline (open circles), FG 7142 (filled circles), CDP (open triangles), CDP plus FG 7142 (filled triangles), Ro15-1788 (open squares), and Ro15-1788 plus FG 7142 (filled squares). Group means which are not linked by the dotted line on the left are significantly different (Tukey, $p<0.05$). S.E.s are less than 15% of mean values.

and aggressive, are presented in lieu of their constituent behaviors. All data are based on the PT measure. Data on BF and BD measures are shown only when these values depart from the pattern of the PT data.

ANOVA produced significant effects for the PT of the following behaviors: locomote-environment trials, $F(3,126)=74.02$, $p<0.01$, locomotion trials, $F(3,126)=140.34$, $p<0.01$, sniff-environment groups, $F(5,42)=4.14$, $p<0.01$, trials, $F(3,126)=18.32$, $p<0.01$, and interaction, $F(15,126)=2.47$, $p<0.01$, groom trials, $F(3,126)=22.82$, $p<0.01$ and interaction, $F(15,126)=1.78$, $p<0.05$, investigative groups, $F(5,42)=5.47$, $p<0.01$, trials, $F(3,126)=10.60$, $p<0.01$, and interaction, $F(15,126)=2.00$, $p<0.05$, avoiding groups, $F(5,42)=5.14$, $p<0.01$, and interaction, $F(15,126)=2.12$, $p<0.05$, aggressive groups, $F(5,42)=20.06$, $p<0.01$, and interaction, $F(15,126)=2.85$, $p<0.01$, and total-social groups, $F(5,42)=4.58$, $p<0.01$, and trials, $F(3,126)=2.78$, $p<0.05$. BF differed from this pattern for PT in yielding significant group effects for locomote-environment, $F(5,42)=5.34$, $p<0.01$, and locomotion, $F(5,42)=3.43$, $p<0.05$. The significant F values for the frequency of pins were groups, $F(5,42)=9.93$, $p<0.01$, trials, $F(3,126)=13.98$, $p<0.01$, and interaction, $F(15,126)=2.50$, $p<0.01$.

Tukey test results for within trial comparison of the groups are presented for selected behaviors in Figs. 1–3. Within group comparison of trial scores by the Tukey test are not presented in the figures although the test results are discussed.

The saline group declined over trials in BF of locomote-environment and locomotion (Fig. 1) and declined in PT of sniff-environment (not shown), while increasing the PT of groom (not shown). The PT of social activity (total-social) of the saline rats did not change because, whereas their PT of aggression declined across trials, their PT of avoiding increased and their PT of investigative behavior did not change (Fig. 2). Their frequency of pins declined over trials along with their level of aggressive behavior (Fig. 3).

The animals treated with chlordiazepoxide alone behaved similarly to the saline-treated animals. The only difference was that the PT of aggressive behavior did not decline over trials and the PT of avoiding did not increase over trials as did that of the saline rats (Fig. 2).

Compared to saline rats, FG rats showed increased PT of investigative behavior (Fig. 2), decreased PT of aggressive behavior (Fig. 2), decreased frequency of pins (Fig. 3), and increased PT of avoiding behavior (Fig. 2). All of these FG

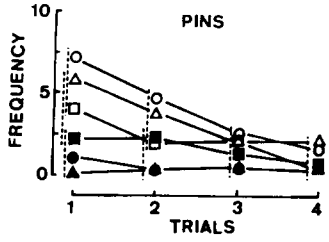

FIG. 3. Mean frequency of pins over 4 trials for 6 groups (n=8) saline (open circles), FG 7142 (filled circles), CDP (open triangles), CDP plus FG 7142 (filled triangles), Ro15-1788 (open squares), and Ro15-1788 plus FG 7142 (filled squares). Group means which are not linked by the dotted line on the left are significantly different (Tukey, $p<0.05$). S.E.s are less than 15% of mean values.

effects occurred in the first two trials, except decreased aggression which extended over three trials (Fig. 2). The PT of total-social behavior of the FG rats was not significantly different from that of the saline rats (Fig. 2).

The rats given chlordiazepoxide plus FG 7142 did not behave differently from rats treated with only FG 7142 (Figs. 1–3). Indeed the pattern of significant differences between the CDP-FG and saline rats was similar to that of the differences between the FG and the saline rats. Compared to saline-treated rats, the CDP-FG animals increased their PT of investigative behavior (Fig. 2) decreased their PT of aggressive behavior (Fig. 2), decreased their frequency of pins (Fig. 3) and increased their PT of avoiding behavior (Fig. 2). All of these effects occurred within the first two trials of the session.

The Ro15-1788-treated rats were not significantly different from saline control rats in direct comparisons of the two groups by Tukey tests (Figs. 1–3). Figure 1 shows data for both locomote-environment and locomotion because although the former behaviour is more revealing of Ro15-1788 effects, the latter is a closer approximation to the most frequently reported measure of ambulation [4]. The Ro rats however, did have a pattern of significant differences with the FG and CDP-FG groups which was different from that shown by the saline rats. Ro rats compared to FG and CDP-FG rats showed increased PT and BF of locomote-environment (Fig. 1), increased BF of locomotion (Fig. 1), and increased PT of sniff-environment (not shown), whereas these comparisons between saline versus FG and CDP-FG rats were not significant (Fig. 1). Inspection of the data (Fig. 1) revealed that this pattern of differences occurred because the scores of the saline rats fell between those of the Ro rats on the one hand and those of the FG and CDP-FG rats on the other. The same pattern in the results appeared in the social behaviors.

Compared to FG and CDP-FG rats, the Ro rats exhibited decreased PT of investigative behavior (Fig. 2), decreased PT of avoiding behavior (Fig. 2), and increased PT of aggression (Fig. 2). In addition Ro rats evinced decreased PT of total-social behavior compared to FG and CDP-FG rats (Fig. 2).

Rats treated with Ro15-1788 plus FG 7142 did not differ on direct comparison with rats treated with Ro15-1788 alone (Figs. 1–3). In contrast Ro-FG rats differed from FG and CDP-FG rats in a manner similar to Ro rats. Relative to FG and CDP-FG rats, rats treated with Ro 15-788 plus FG 7142 increased BF of locomote-environment (Fig. 1), increased PT of sniff-environment (not shown), decreased PT of investigative behavior (Fig. 2), increased PT of aggressive behavior (Fig. 2), decreased PT of avoiding behavior (Fig. 2), and decreased PT of total-social behavior (Fig. 2).

In summary, the overall picture is that of the mean scores of the Ro and Ro-FG rats on one extreme, the scores of the FG and CDP-FG rats on the other extreme and the scores of the CDP and saline rats in the middle.

DISCUSSION

The present study confirms an earlier report [1] that FG 7142 decreased aggressive behavior and increased avoiding behavior without changing total-social behavior of male rats tested in pairs. The present results showed that the effect is generalizable from rats housed together to those housed individually. This is an important point for external validation because isolation housing compared to group housing produces increased active social contact in rats [12–13, 18] and increased aggression in mice [11,16]. Finally, as noted in the introduction, FG 7142 has been reported to decrease aggression in female rats defending nursing litters against intruding males [8]. In sum FG 7142 decreases conspecific aggression in adult rats in several situations.

An acute dose of CDP had little effect compared to saline on exploratory or social behavior of animals tested during the initial 20 min of being together ([5, 17, 23], present study). The present study is the first to demonstrate that whereas the aggressive behavior of saline-treated animals declines after 20 min of social interaction, that of the CDP treated animals does not.

Chlordiazepoxide failed to reverse the effects of FG 7142 contrary to expectations based on a social interaction test [6] and on an intracranial self-stimulation test [20]. The social interaction test was administered for 7.5 min, during the postinjection interval equivalent to the third trial of the present study. Our data show that whereas CDP had no effect on aggression before the third trial, subsequently CDP tended to accelerate the recovery of the FG 7142-treated rats. Thus the partial CDP reversal of FG 7142-induced effects in the present study is consistent with that of the social interaction test [7], if the time course is considered. A greater magnitude of CDP reversal might have been achieved had a higher CDP dose been injected.

Ro15-1788-treated rats compared to saline-treated rats showed marginally increased locomotion and sniff-environment and decreased total-social behavior in support of reports of Ro15-1788-induced increases in rearing and locomotion in mice [21–22] and decreased social interaction in rats [6]. The present results permit generalization of the Ro15-1788-induced suppressive effects on social behavior from the largely affiliative social interaction test [6] to the present test involving more aggression and avoidance than affilitative behavior.

Ro15-1788 pretreatment reversed the effects of FG 7142 on total-social behavior as has been reported in the social interaction test [6]. Such a reversal has also been reported in a punished drinking paradigm [3]. The present study is the first to show specifically that the FG 7142 effects on aggressive and avoidance behavior are also reversed by Ro15-1788.

Considered in conjunction with observations by ourselves (present study) and others in several species [4, 7, 14] that benzodiazepine treatment increases aggression, the Ro15-1788 reversal of the FG 7142-induced suppression of aggres-

sive behavior supports the hypothesis that the anti-aggressive effects of FG 7142 are mediated by benzodiazepine receptors. The failure of acute chloridazepoxide pretreatment to reverse the FG 7142 effect is perhaps the result of the lower rank of chlordiazepoxide, a benzodiazepine receptor agonist relative to that of Ro15-1788, a benzodiazepine receptor antagonist on the pharmacological scale of efficacy of benzodiazepine receptor ligands [2–3, 10]. The behavioral specificity of the FG 7142 effects on agonistic behavior ([1,7], present study) argues against a global FG 7142 effect mediated by neurotoxicity or prodromal seizure activity.

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