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Stressor Controllability, Social Interaction, and Benzodiazepine Systems

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SHORT, K. R. and S. F. MAIER. *Stressor controllability, social interaction, and benzodiazepine systems.* PHARMACOL BIOCHEM BEHAV **45**(4) 827–835, 1993.—Effects of benzodiazepine receptor-active compounds on inescapable shock-produced changes in social interaction were studied in the rat. Inescapably shocked animals exhibited less social interaction in a novel situation than did escapably shocked or unshocked rats 24 h after shock. Administration of the selective benzodiazepine receptor antagonist flumazenil at the time of shock prevented the decrease in social interaction. Social interaction was unaffected by the same treatment at the time of measurement. Reduction in social interaction induced by inescapable stress endured for 48–72 h following stressor exposure but was absent 168 h after stress. It was subject to antagonist blockade at all measured time points. Stress-induced decreases in social interaction were also blocked by the benzodiazepine chlordiazepoxide given at the time of shock treatment. The receptor antagonist did not reverse this blockade. An inverse agonist, the β-carboline FG 7142, administered in place of inescapable shock, produced an identical pattern of social interaction in a dose-dependent manner. The inverse agonist effect was also reversed by the antagonist. The results from antagonist, agonist, and inverse agonist treatments all suggest that an endogenous benzodiazepine receptor inverse agonist is released at the time of inescapable shock and is involved in producing the changes in social interaction subsequently measured.

Learned helplessness Stress Anxiety Inescapable shock Social interaction Benzodiazepine receptor
Endogenous ligands Flumazenil Ro15-1788 FG 7142 Chlordiazepoxide Rat

THE control that an organism has over a stressor (the ability to alter the onset, termination, duration, intensity, or temporal pattern of the stressor) exerts a strong modulatory effect on the behavioral consequences of exposure to the stressor [see (25) for a recent review]. A variety of behavioral changes follow exposure to stressors such as electric shock if the shocks are inescapable, but do not if the shocks are escapable. Effects such as these, which depend on the controllability of the stressor, have been called "learned helplessness effects" (26).

The most intensively studied outcome is shock escape learning. Exposure to inescapable shock, but not to equal amounts of escapable shock, often interferes with escape learning in a different situation (30). However, the impact of stressor controllability extends to many other subsequent behaviors. A broad range of behaviors is altered by inescapable shock. Unconditioned activity in reaction to aversive events such as shock or water, perseverative response tendencies, shifts in attention away from internal toward external cues, shock-elicited aggression, defensive burying, aggressive attack against an intruder, defensive aggression, dominance in competition for food, reductions in food intake in response to bitter tastes, "opioid" analgesia, and pup retrieval from a nest are but a partial list [see (25) for a review].

Any explanation of how inescapable shock might produce such a diverse set of results would have to place emphasis on an underlying process that is itself capable of affecting a broad range of behaviors. An attractive hypothesis revolves around conditioned fear and fear-related behaviors. Conditioned fear is most often measured by the occurrence of freezing (1) or the suppression of food or water intake. Fear becomes conditioned both to contextual stimuli present during the occurrence of aversive events such as shock and to discrete stimuli that signal the shocks. Importantly, more fear becomes conditioned to cues present during shock if the shocks are inescapable than if the shocks are equal but escapable (4,29). This is presumably because inescapable shock produces more fear, and, therefore, more fear is conditioned to stimuli that are present.

Enhanced fear could be responsible for the later behaviors through a process of transfer of conditioned fear. Perhaps there are stimuli present in the test environments that are sufficiently similar to those that had been present in the original shock situation that conditioned fear transfers or generalizes to the new test situation. Because more fear was conditioned in inescapably than in escapably shocked animals, there would be more fear available to transfer. High levels of conditioned

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fear in the test environment might then lead to the behavioral changes observed. For example, Williams and Lierle (39) have argued that this fear produces both a) freezing which can interfere with other behaviors such as active escape, defensive burying, aggression, and so on, and b) a motivational state which can interact with the normal motivation produced in the task to alter the normal behavioral reactions that occur.

There are data to support this hypothesis. Reducing the amount of fear during the inescapable shock exposure, either by pharmacological (8) or behavioral (36) means, reduces or eliminates the escape learning deficit that otherwise follows. However, there are several difficulties for the hypothesis. The first concerns the time course of learned helplessness effects. The behavioral changes described above have been typically assessed 24 h after the shock exposure. In those cases where this interval has been manipulated, the effects disappeared if 48–72 h intervened between inescapable shock and testing. The time interval between inescapable shock and testing has been manipulated in studies of shuttlebox escape learning (23), activity in response to shock (23), freezing after exposure to shock in a new environment (24), analgesia upon reexposure to shock (22), shock-elicited aggression (Maier, unpublished), and struggling when placed in water (38). In all these cases the behavior was present 48 h, but not 72 h after inescapable shock.

However, conditioned fear is not a transient phenomenon that dissipates rapidly. Conditioned fear responses are extremely resistant to dissipation with time (forgetting), and remain undiminished even after intervals of weeks to months (21). This resistance to loss over time is observed even after just a few pairings of a stimulus with shock (19), and so it is implausible that the fear conditioned to cues present during the 80–100 inescapable shocks used in the learned helplessness experiments is forgotten in 72 h, but not in 48 h.

A second difficulty for the transfer of conditioned fear hypothesis is that subjects given inescapable shock in one situation (a wheel-turn box) show very little fear when placed in the typical shuttlebox test task 24 h later (24). In sum, the fear aroused during inescapable shock would seem to be important, but the transfer of conditioned fear would not seem to be a viable mechanism. The time course suggests an unconditioned rather than a conditioned change. It suggests that inescapable shock sensitizes a fear process, a change that dissipates over a 48–72 h interval. To test this idea, Maier (24) gave rats one or two footshocks in a shuttlebox 24 h after either escapable or inescapable tail shocks administered in the wheel-turn apparatus. These shocks produced freezing in all subjects, but the freezing that developed was exaggerated in the inescapably shocked rats. This enhancement of the freezing produced by shock was no longer present if 72 h intervened between the original shock exposure and testing.

The "sensitized fear process" may be what is normally referred to as "anxiety," as distinct from conditioned fear. Conditioned fear is a response that has an identifiable target and is "appropriate" to the degree of danger. It is tied to a stimulus that can produce directed defensive behavior (9). Anxiety, on the other hand, is not tied to a clearly threatening stimulus (a predator, a tone that signals shock, etc.) and represents either fear that is out of proportion to any threat or an exaggerated response to an ambiguous situation (20).

Thus it might be that uncontrollable aversive events precipitate anxiety that is still present 24 h later, and that perhaps dissipates over a 48–72 h interval. The purpose of the present experiments was to determine whether inescapable shock, as opposed to equal amounts of escapable shock, leads to behavioral changes (putatively related to anxiety) that are still present 24 h later. Two strategies were possible in this intitial set of studies. One was to explore a number of different behavioral tests in preliminary fashion that are thought to reflect "anxiety," with the goal of determining whether there is a consistent effect across tests. The second was to select a single test, but to investigate any effects that occured in detail. The latter strategy was chosen.

Two types of behavioral tests have been argued to reflect anxiety [see (2) for a review]. One establishes a conflict, and examines the degree of behavioral suppression produced. The second exposes the subject to a novel or mildly threatening environment, and focuses on suppression of behavior that normally occurs in the situation. The conflict procedures involve the use of shock and food or water reward. Inescapable shock alters both pain sensitivity (22) and food and water intake (37). Thus any potential results using the conflict procedures would be confounded and difficult to interpret. We, therefore, chose a test from the second category. For these initial experiments we selected the social interaction test (12) because it has been extensively validated [e.g., (10)]. Two rats are placed together in a novel arena and their behavior measured. Manipulations known to increase anxiety in humans decrease the level of social interaction (see below for definition) observed, while manipulations known to decrease anxiety increase interaction.

Experiment 1 explored whether escapable shock or equal amounts of inescapable shock delivered in wheel-turn boxes would alter the level of social interaction measured 24 h later. The benzodiazepine-$GABA_A$ receptor/chloride ionophore complex is thought to play a critical role in modulating anxiety [see (5) for a review] and has been shown to be of importance in mediating some of the behavioral effects of inescapable shock (8,31). Experiments 2–5, therefore, examined the ability of agents that act on this complex to block or mimic any effect of inescapable shock on social interaction 24 h later. Experiment 2 determined whether the benzodiazepine (BZ) chlordiazepoxide (CDP) would alter the impact of either escapable or inescapable shock when administered before the shock exposure, and whether any such effect would be prevented by the putative BZ receptor antagonist flumazenil (Ro15-1788). Experiments 3 and 4 further examined the impact of flumazenil by itself. Experiment 3 investigated whether flumazenil given either before the shock or before the social interaction testing would change the effect of escapable or inescapable shock. Experiment 4 manipulated the dose of flumazenil under the conditions where it had been effective in Experiment 3. Experiment 5 sought to determine whether the anxiogenic BZ receptor inverse agonist FG 7142 would mimic the effect of inescapable shock by reducing social interaction 24 h later. FG 7142 is known to reduce social interaction shortly after administration (15), but whether it has a more extended effect that outlives its presence at the receptor is unknown. Experiment 6 examined the time interval across which inescapable shock might influence subsequent social interaction.

METHOD

Animals

The subjects in all experiments were male Sprague-Dawley rats obtained from SASCO and housed singly for at least 5 days prior to use. They were maintained on a 12L : 12D cycle and trained and tested during the light phase of the cycle.

Food and water were continuously available. Subjects weighed between 180 and 300 g.

Apparatus

Inescapable shock, escapable shock, or restraint occurred in Plexiglas wheel-turn chambers measuring 14 × 11 × 17 cm. The Plexiglas wheel was 9 cm wide and 14 cm diameter and extended 3 cm into the chamber through the front 11 × 17 cm panel. The curved surface of the wheel consisted of 14 stainless steel rods, each 8 cm long and 0.6 cm diameter, arranged parallel to one another and to the axis of rotation and spaced at equal intervals around the circumference of the wheel. The rat's tail extended through an opening in the rear wall of the chamber and was attached with adhesive tape to a Plexiglas bar that extended 6 cm beyond the rear wall of the chamber. Fuse clip electrodes were coated with electrode paste and attached with adhesive tape to the tail, approximately 3 cm from the base of the tail. Constant-current, unscrambled shock was delivered from a shock source modeled after a Grason-Stadler Model 700. Masking noise and ventilation were provided by cooling fans adjacent to each wheel-turn chamber.

Social interaction testing occurred in a wooden box 60 × 60 cm with black walls 50 cm high. The enamelled white floor was evenly illuminated from a distance of 1.5 m by four 60 W/120 V incandescent reflector lamps. The floor was marked with black lines to form 16 squares, each 15 × 15 cm. Activity was videotaped by a camera suspended 1.5 m above the arena floor and was viewed from an adjacent room.

Drugs

Chlordiazepoxide (Sigma), flumazenil (Ro15-1788, a generous gift from Hoffmann-La Roche), and FG 7142 (N-methyl-β-carboline-3-carboxamide, RBI) were all injected IP, dissolved or ultrasonically dispersed in saline and Tween 80 (polyoxyethylene sorbitan mono-oleate, Sigma). Two drops of Tween per 10 ml of solution were used.

Statistics

The data were analyzed by analysis of variance. Post hoc comparisons were made by Newman-Keuls tests ($p < 0.05$).

Procedure

In experiments where shock controllability was manipulated, rats were treated in sets of three (one from each shock condition: escape, yoked, or restrained). The escape rat in each set received 100 trials of unsignalled 1.0 mA shock on a VI 60-s schedule (range: 30–90 s). After the initial 0.8 s of shock not under subject's control, shock could be terminated by the appropriate wheel-turn response. A variable response requirement was implemented to delay the appearance of asymptotic performance and to maintain an average shock duration of approximately 5–10 s. The initial response requirement was one 90° turn of the wheel, the basic unit of response that was measured. Subsequent response requirements were increased according to prior response latencies. Three consecutive response latencies under 5.0 s caused a one-unit increase in the response requirement for the following trial. A latency under 5 s on that trial resulted in a two-unit increase for the next trial; each subsequent response latency under 5.0 s resulted in a doubling of the previous increment in response requirement, up to a maximum response requirement of 16 units or four complete rotations of the wheel.

Any interruption of the increment sequence by response latencies over 5.0 s caused the sequence to restart with a requirement of three consecutive rapid-response trials. Response latencies of 10–29 s would decrement the response requirement for the next trial by one unit; failure to complete a response, or a response latency of 30 s, the maximum shock duration allowed, reset the response requirement to one response unit. Response latency was measured from shock onset to the completion of the response requirement. ITI was measured from response completion to shock onset.

The second rat in each set of three was inescapably shocked. Shock was terminated for the second (yoked) rat only by responses made by the first (escape) rat in the set. Both rats thus received the same number, pattern, intensity, and duration of shocks. Responses made by yoked subjects had no effect on shock termination or onset.

The third rat in each set was restrained in an identical chamber for the same duration, and electrodes were attached as described for the other two rats, but no shock was delivered.

In experiments that compared only inescapable shock with restraint, the inescapably shocked animals received a pattern and duration of inescapable shocks typical of that provided to yoked subjects paired with escape subjects. The exact trial by trial durations that occurred in the first escape-yoked experiment (Experiment 1) were recorded by computer and simply used to determine the durations of each shock. Thus the pattern of shocks was the same for rats that received inescapable shock as part of a comparison with only restrained controls and as part of an escape-yoked comparison.

In social interaction testing, two rats that had received identical treatments on the previous day were placed in the center of the arena and their actions were recorded for a 10-min period. Two independent observers, one of whom was blind to treatment condition, measured: a) the total time spent in active social interaction, and b) the number of crossings of boundaries of the 15-cm squares. The behaviors deemed active interactions consist of: sniffing, following, grooming, kicking, pushing, standing on, wrestling or boxing with, crawling over or under, or mounting the partner. The amount of time spent passively lying on one another was not scored. The time engaged in these activities was measured for the pair; no individual score was assigned. Locomotion scores for each 10-min session represented the movement of one randomly chosen member of each pair. Order of testing within sets of rats (across shock condition) was counterbalanced across days of testing.

Experiment 1. Controllability

Sixty rats were randomly assigned to receive either escapable shock ($n = 20$), yoked inescapable shock ($n = 20$), or restraint ($n = 20$). One hundred shocks were delivered at 1.0 mA intensity, on a VI 60-s schedule. Pairs of similarly treated rats were tested for amount of social interaction 24 h later.

Experiment 2. CDP

Two hundred forty rats were divided between escapable shock, yoked inescapable shock, or restraint on day 1. Social interaction testing occurred 24 h later, on day 2. Pairs to be tested were always formed from identically treated subjects. The day 1 treatment was preceded by 2 IP injections, one immediately after the other. For half of the animals the first injection was 15 mg/kg CDP and for the other half vehicle. For half of each of these groups the second injection was

vehicle, and for the other half it was 10 mg/kg flumazenil. Experimental treatment followed injection by 10 min. Rats that received CDP were given a single administration of 15 mg/kg CDP each day, for the 4 days preceding day 1 of the experiment. The purpose of this procedure was to "tolerate out" potential sedative and ataxic effects (11). This is the procedure used by Drugan et al. (8) and Maier (24). The vehicle subjects were given vehicle on these 4 days. Thus, the design of this experiment was a 3 (escapable shock, yoked inescapable shock, restraint) × 2 (CDP, vehicle) × 2 (flumazenil, vehicle) factorial.

Experiment 3. Flumazenil

Two hundred sixteen rats were divided between escapable shock, yoked inescapable shock, or restraint on day 1. Social interaction testing between identically treated pairs occurred on day 2. Each rat received 10 mg/kg flumazenil or an equivolume injection of vehicle 10 min prior to treatment or 10 min prior to social interaction testing. No animals received flumazenil before both training and testing. There were thus 3 levels of drug treatment: drug before training and vehicle before testing, vehicle before training and drug before testing, and vehicle before both training and testing. The overall design was thus a 3 × 3 factorial, with 24 rats in 12 pairs providing scores for each of the nine conditions.

Experiment 4. Flumazenil Dose-Response

One hundred sixty rats were divided between inescapable shock and restraint conditions. Social interaction testing occurred 24 h later. Ten minutes prior to day 1 treatment the rats received either 0, 5, 10, or 25 mg/kg flumazenil. Escapable shock was not employed because Experiment 3 found an effect of flumazenil only on the inescapably shocked subjects, and here we wished to examine whether this effect was dose dependent.

Experiment 5. FG 7142

Two hundred forty rats received two successive IP injections 10 min before restraint of the usual duration, and one injection 10 min prior to social interaction testing 24 h later. One injection before day 1 restraint was either 0, 5, 10, or 20 mg/kg FG 7142. The other was either vehicle or 10 mg/kg flumazenil. The injection prior to day 2 interaction testing was either vehicle or 10 mg/kg flumazenil. The design was not fully a 4 × 2 × 2 factorial, however, because no rats received flumazenil on both days. No useful information could have been obtained from such a group. Thus within each of the four FG 7142 dose conditions there were three further conditions: vehicle before FG 7142 and vehicle before testing, vehicle before FG 7142 and flumazenil before interaction testing, and flumazenil before FG 7142. This yields an overall 4 × 3 factorial design with 20 rats providing 10 data points for each of the 12 groups.

Experiment 6. Time Course

Four hundred rats were divided between inescapable shock or restraint on day 1. Half of each of these groups received 10 mg/kg flumazenil, and half equivolume vehicle 10 min before treatment. Social interaction testing occurred either 2 h, 24 h, 48 h, 72 h, or 168 h after treatment, with 1/5 of each group tested at each interval. Thus the design is a 2 (inescapable shock, restraint) × 2 (flumazenil, vehicle) × 5 (2-, 24-, 48-, 72-, or 168-h interval between treatment and test) factorial, with 10 pairs in each condition.

RESULTS

Shock Controllability

Figure 1 shows the mean amount of social interaction and locomotion for each of the groups in Experiment 1. As can be seen, there was little, if any, effect of escapable or inescapable shock on locomotion. Escapable shock did not alter social interaction. However, inescapably shocked subjects showed reduced levels of interaction. ANOVA yielded a reliable effect of groups with regard to social interaction, $F(1, 27) = 6.36$, $p < 0.05$, but not with regard to locomotion. Subsequent Newman-Keuls comparisons found the yoked group to differ from both the escape and restrained groups, which did not differ from each other.

The correlation between interaction and locomotion scores ($r = 0.24$) was not reliable. However, because extremes of locomotion levels could affect the amount of interaction, all interaction scores were corrected for the amount of locomotion. An adjusted interaction score was computed for each subject by dividing level of interaction by amount of locomotion. Figure 2 shows the adjusted social interaction data. An ANOVA applied to these data yielded a reliable difference between groups, $F(1, 27) = 5.81$, $p < 0.05$, and Newman-Keuls tests found the yoked group again to differ from each of the other two, which did not differ from each other. Adjusted interaction scores will be presented in the remainder of this paper because of possible effects of drug or shock treatments on activity. However, in no case would conclusions be altered by examining uncorrected interaction data.

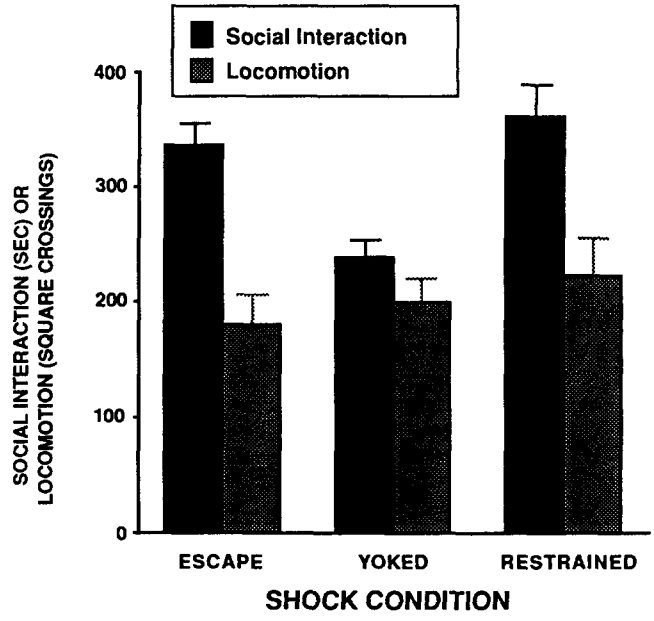

FIG. 1. The effect of type of shock treatment on social interaction and locomotor activity. Means of social interaction scores (s) and locomotion scores (square crossings) ± SEM are plotted as a function of type of shock treatment.

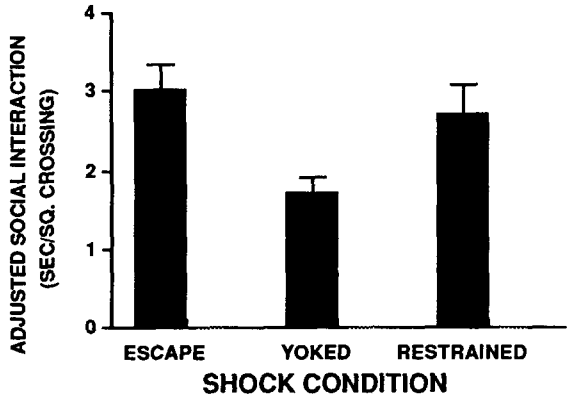

FIG. 2. The effect of type of shock treatment on adjusted social interaction. Means of the adjusted social interaction scores (in s of interaction/squares crossed) ± SEM are plotted as a function of the type of shock treatment.

CDP

The adjusted social interaction scores for Experiment 2 are in Fig. 3. As in Experiment 1, yoked inescapable but not escapable shock led to reduced interaction 24 h later. CDP administered before shock completely blocked this effect. Interestingly, flumazenil also blocked the reduction in social interaction when administered before inescapable shock, and the combination of CDP and flumazenil also led to a blockade. Neither CDP nor flumazenil had any effect on the interaction scores of escapably shocked subjects or restrained controls tested 24 h later. A two-way between-subjects ANOVA yielded a reliable group × drug interaction, $F(6, 108) = 2.73$, $p < 0.05$. Subsequent Newman-Keuls tests found the yoked group that had received only vehicle to differ from all the others, which did not differ among themselves.

Flumazenil

The effect of flumazenil in Experiment 2 prompted an investigation of flumazenil by itself. The results of the flumazenil experiment (Experiment 3) are in Fig. 4. As before, inescapable but not escapable shock led to reductions in social interaction 24 h later. As in Experiment 2, flumazenil administered before inescapable shock blocked this effect of inescapable shock. However, flumazenil did not alter the effect of inescapable shock when it was administered before the social interaction test. Flumazenil had no effect on social interaction in the other groups, whether administered before the social interaction test or before treatment 24 h earlier. ANOVA yielded reliable effects of group $F(2, 99) = 4.41$, $p > 0.05$, drug, $F(2, 99) = 3.19$, $p < 0.05$, and their interaction, $F(4, 99) = 3.29$, $p < 0.05$. Newman-Keuls tests showed the yoked groups that received only vehicle or flumazenil before the interaction test to differ from the others, which did not differ among themselves.

Flumazenil Dose-Response

The results of the flumazenil dose-response experiment are in Fig. 5. As in the previous experiments, inescapable shock reduced social interaction 24 h later. The lowest (5 mg/kg) dose was not sufficient to block the effect of inescapable

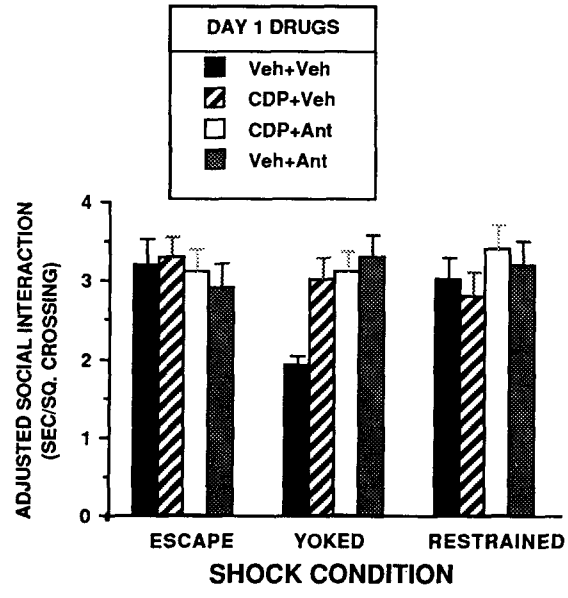

FIG. 3. The effects of shock and CDP and flumazenil on adjusted social interaction. Means of the adjusted social interaction scores (in s of interaction/squares crossed) ± SEM are plotted as a function of type of shock treatment and drug treatment received. "Ant" represents an injection of the antagonist flumazenil, "CDP" represents an injection of the benzodiazepine chlordiazepoxide, and "Veh" represents an injection of the vehicle alone. These injections were combined as indicated at the time of shock treatment. Day 1 injections were preceded by repeated doses of either CDP or Veh as described in the text.

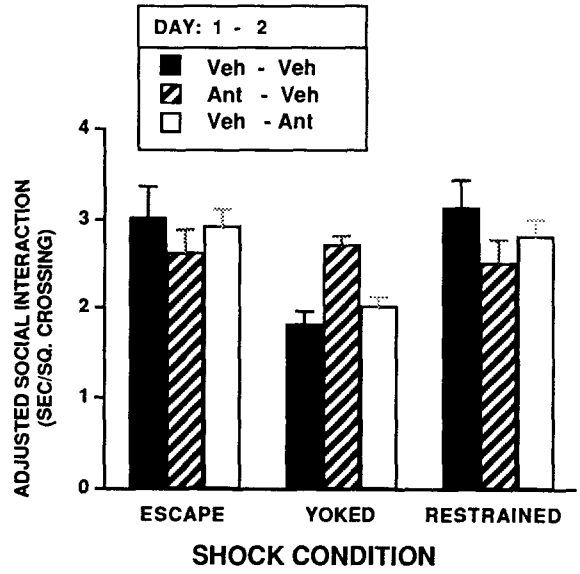

FIG. 4. The effects of type of shock treatment and flumazenil on adjusted social interaction. Means of the adjusted social interaction scores (in s of interaction/squares crossed) ± SEM are plotted as a function of type of shock treatment and drug treatment received. "Ant" represents an injection of the antagonist flumazenil (Ro15-1788) and "Veh" represents an injection of the vehicle alone. Drugs listed before the hyphen were injected before shock treatment, while those after were injected before interaction testing.

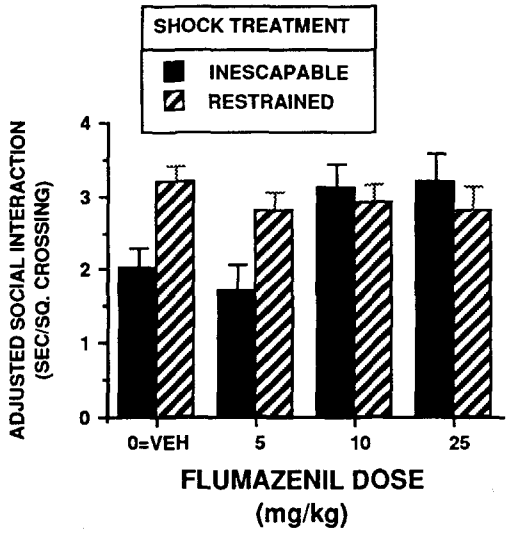

FIG. 5. The effects of shock and dose of flumazenil on social interaction, adjusted for locomotor activity. Means of the adjusted social interaction scores (in s of interaction/squares crossed) ± SEM are plotted as a function of type of shock treatment and amount of flumazenil received. The 0 mg/kg dose represents the nondrug or vehicle control. "Ant" represents an injection of the antagonist flumazenil (Ro15-1788) and "Veh" represents an injection of the vehicle alone. Drugs listed before the hyphen were injected before shock treatment, while those after were injected before interaction testing.

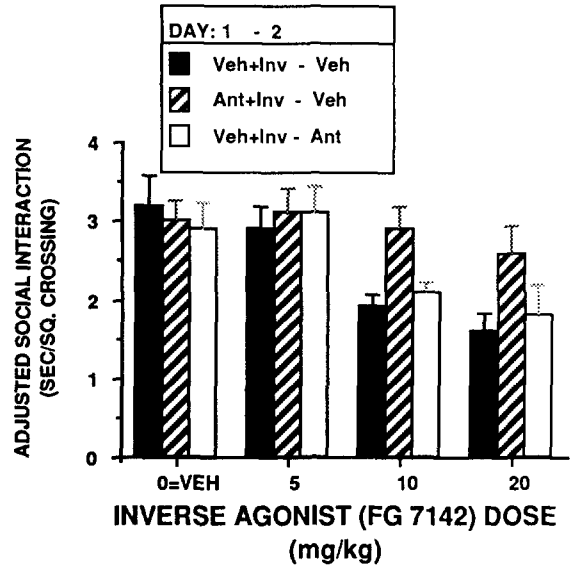

FIG. 6. The effects of FG 7142 dose and flumazenil on social interaction, adjusted for locomotor activity. Means of the adjusted social interaction scores (in s of interaction/squares crossed) ± SEM are plotted as a function of type and amount of drug treatment received. The 0 mg/kg dose served as the nondrug or vehicle control. "Ant" represents an injection of the antagonist flumazenil, "Inv" represents an injection of the inverse agonist FG 7142, and "Veh" represents an injection of the vehicle or solvent alone. Drugs listed before the hyphen were combined as indicated on day 1 in lieu of shock treatment, while those after the hyphen were injected before testing on day 2.

shock when administered before inescapable shock. However, both 10 mg/kg and 25 mg/kg completely blocked the effect of inescapable shock. ANOVA revealed a significant effect of dose, $F(3, 72) = 5.70$, $p < 0.01$, and interaction between groups and dose, $F(3, 72) = 4.77$, $p < 0.01$. Newman-Keuls comparisons indicated that inescapably shocked rats that received 0 or 5 mg/kg flumazenil differed from all of the others, which did not differ among themselves.

FG 7142

The impact of FG 7142 on social interaction 24 h later is depicted in Fig. 6. It is apparent that 10 and 20 mg/kg led to a reduction in social interaction, while 5 mg/kg did not. Moreover, duplicating the pattern with inescapable shock, the effect of FG 7142 was blocked by flumazenil administered at the time of FG 7142 administration, but not at the time of testing.

Time Course

The results of this experiment are shown in Fig. 7. In general, inescapable shock led to a reduction in interaction, and the effect did dissipate with time. This effect was again blocked by flumazenil given before inescapable shock. Restraint tended to reduce social interaction 2 h later, and flumazenil did not reduce this effect of restraint. ANOVA produced a reliable effect of interval, $F(4, 180) = 2.67$, $p < 0.05$, and the interaction of group × drug, $F(1, 180) = 3.95$, $p < 0.05$. Newman-Keuls tests were used to compare each of the groups that had received inescapable shock and vehicle with the other groups tested at that time interval. The inescapably shocked vehicle group differed from the others reliably only at the 24- and 48-h test intervals. The difference was marginally signifi-

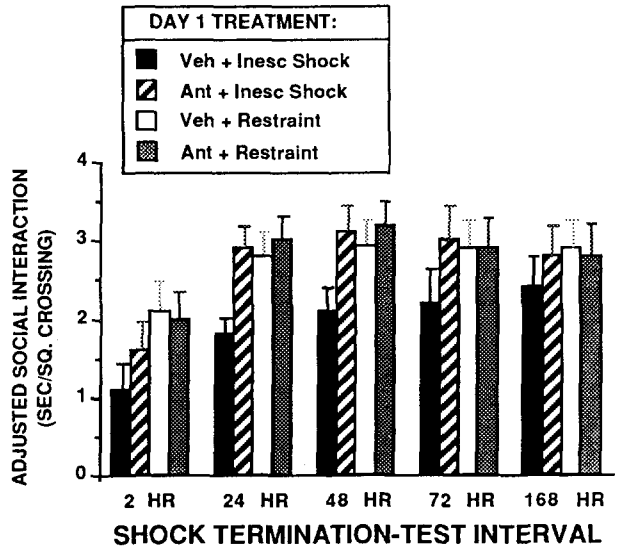

FIG. 7. Time course of the benzodiazepine receptor mediated effect of shock on social interaction, adjusted for locomotor activity. Means of the adjusted social interaction scores (in s of interaction/squares crossed) ± SEM are plotted as a function of type of shock treatment and latency between treatment and testing. "Ant" represents an injection of the antagonist flumazenil and "Veh" represents an injection of the vehicle alone. Injections were given at the time of treatment only.

cant ($p < 0.10$) at 72 h. It did not approach significance at 168 h.

DISCUSSION

The results of this set of experiments clearly demonstrate that shock controllability is an important factor in producing a reduction in social interaction that a) persists beyond the period of shock exposure and b) is measurable in a very different situation. Inescapably shocked animals interacted less than did controls 24 h after inescapable shock, while escapably shocked rats showed no such reductions. It might be noted that the behavior of the animals was carefully observed, and the test situation did not lead to overt signs of conditioned fear such as freezing. Furthermore, the reduction in interaction was not a product of reductions in locomotion. The inescapably shocked animals did not respond to the test situation with freezing or inhibited movement; they simply interacted less with each other.

The time course of this effect was similar to that observed for other behavioral sequelae of inescapable shock. The effects of inescapable shock on shuttlebox escape have been reported to require several hours after shock to develop (18). This was also the case here, although it must be noted that a change in the control group rather than in the inescapably shocked group was the factor responsible. Restraint itself appeared to have a brief effect on interaction, leading to the absence of a difference between inescapably shocked animals and controls at 2 h. It is unknown whether other behavioral effects of inescapable shock (e.g., alterations in aggression, defensive burying, activity, etc.) require time to develop, and so it is not clear whether this time point is of importance. More critically, the effect of inescapable shock was no longer reliable at a 72-h shock-to-test interval, and was completely absent at 168 h. This matches the time course of other consequences of inescapable shock that have been studied.

Two general interpretations of the initial controllability effect are possible. Shock itself may induce anxiety because it is an aversive event; learning that the shock is controllable may then reduce the level of anxiety produced by the shock. Alternatively, perhaps learning that the shock is uncontrollable increases anxiety. The two interpretations are not incompatible. That is, control might be anxiolytic, lack of control anxiogenic, or both. Whether the state or process that underlies level of social interaction is called "anxiety" or something else is not important here. The issue is whether shock produces the state or change that is then reduced by control, or whether lack of control produces or increases it.

Such effects, whether they are labeled anxiety or not, need not be mediated by the BZ receptor, but the data are consistent with the idea that the BZ receptor plays a part. Perhaps shock controllability influences an endogenous ligand for this receptor. Although controversial, there is evidence for both anxiolytic [e.g., (28,34)] and anxiogenic [e.g., (3,27)] endogenous ligands.

The present data have definite implications for the type of ligand that would be required. The fact that CDP blocked the reduction in social interaction when administered before the shock is not decisive. Either the anxiolytic or the anxiogenic position can predict this result. That FG 7142, like shock, reduced subsequent social interaction may support the interpretation that an anxiogenic ligand is produced by inescapable shock, but such support is weak. However, the BZ receptor antagonist flumazenil produces less ambiguous results. If having control releases an endogenous anxiolytic, then flumazenil should increase anxiety in the escapably shocked animals and have no effect on the inescapably shocked animals. Both groups should show high levels of anxiety. In contrast, if having no control activates an anxiogenic process that acts at the BZ receptor, then flumazenil should decrease anxiety in the inescapably shocked animals. The results clearly demonstrated that flumazenil had no effect on escapably shocked animals or on restrained controls, but it prevented the increased anxiety produced by inescapable shock. This result was replicated in four experiments. Furthermore, flumazenil administered along with CDP produced results no different than CDP alone. The antagonist and the agonist produced the same blockade of shock-induced interaction reductions, perhaps by interfering in a similar manner with the action of an anxiogenic ligand. Thus the present data can be taken as behavioral support for the existence of an endogenous inverse agonist for the BZ receptor.

A potential difficulty concerns the status of flumazenil. It has sometimes been argued that flumazenil is not a pure antagonist (14). However, flumazenil has rarely been reported to have intrinsic effects at the 10 mg/kg dose used here [e.g., (16)], and it had no effect on social interaction by itself, even when administered 10 min before the social interaction test. The intrinsic activity sometimes reported at high doses has usually been that of an inverse agonist (13), a circumstance that would work against the results here obtained. Furthermore, if flumazenil were acting as an inverse agonist, then CDP and flumazenil combined would be expected to diminish the effects of one another. Such an outcome is contrary to that observed in Experiment 2.

The possibility that inescapable shock releases or activates an endogenous BZ inverse agonist is consistent with the results of several other experiments. For example, Drugan, McIntyre, Alpern, and Maier (6) examined the impact of prior escapable shock, yoked inescapable shock, and restraint, on bicucculline-induced seizures. GABA and BZ receptor agonists block or reduce such seizures, while inverse agonists potentiate seizures. Exposure to electric shock per se interferes with seizures by releasing GABA (35), but amounts of inescapable shock sufficient to produce learned helplessness effects potentiate the seizures (6). Furthermore, there is a reduction in the amount of GABA agonist-induced chloride ion flux into synaptosomes taken from inescapably shocked animals (7). This effect on chloride flux is similar to that produced by BZ receptor inverse agonists.

Flumazenil has rarely been reported to block the consequences of exposure to stressors. Social defeat is an exception. Rodgers and Randall (32,33) found flumazenil to dose dependently block the analgesia produced by social defeat. Numerous commonalities between the behavioral and neurochemical effects of inescapable shock and defeat have been noted (17), and they may produce a similar underlying state (39). Thus there may be something relatively unique about inescapable shock and defeat in producing a persisting state of anxiety after an acute exposure.

Flumazenil did not reduce the effect of inescapable shock on social interaction when it was administered before the interaction test, rather than before the inescapable shock. This is consistent with results reported by Drugan and colleagues (8) and Maier (24). In these studies the administration of CDP or diazepam before testing had no effect on the deficit produced by inescapable shock in shuttlebox escape learning, even though both benzodiazepines were effective when administered before the inescapable shock. This suggests that any BZ/GABA receptor-mediated anxiogenic process that is initi-

ated by inescapable shock leads to a subsequent alteration in another neurotransmitter system, which is itself responsible for the behavioral changes observed later. The $GABA_A$ receptor is involved in the inhibition of many neural systems, and so any interference with GABAergic inhibition, as would be produced by an anxiogenic inverse agonist, could modulate many systems.

The present data, however they are interpreted, indicate that inescapable shock sensitizes a process that dissipates with time and which predisposes the organism to react with behavior that others have argued is characteristic of anxiety. This process appears to involve the $BZ/GABA_A$ receptor complex. It remains for future work to determine whether these results will generalize to other putative behavioral tests of anxiety, or whether they are specific to social interaction. The precise mechanism involved and the role of this anxiety-like state in determining the numerous behavioral consequences of uncontrollable stressors remains to be explored.

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