Conditioned fear and analgesia to conspecific odors: Benzodiazepine and 5-HT_{1A} agonists

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When male albino rats experience one session of attack and defeat by an alpha conspecific, conditioned fear and analgesia are observed during later testing only when alpha-colony odors are present. In Experiment 1, subjects experienced a nondefeat (ND) or a defeat (D) session 24 h prior to testing of fear using the shock-prod burying paradigm. Thirty min before ND and D sessions, subjects were injected with saline (SAL), diazepam (DZP; 2.5 mg/kg), or buspirone (BUS; 5 mg/kg). The results indicated that defeated subjects that had been injected with DZP or BUS failed to show the typical decrease in burying and the increase in freezing indicative of the conditioning of fear to the odor context. In Experiment 2 we utilized the same defeat and drug administration procedure but used the formalin paw-lick test of analgesia. The administration of DZP or BUS prevented the conditioning of fear and analgesia to the alpha-colony odors. These are the first experiments to demonstrate that both diazepam and buspirone can block the conditioning of fear and analgesia resulting from the naturalistic stressor of defeat.

An ethological example of conditioned fear results from exposing rats to a defeat session by a larger "alpha" rat of the same species, with odors associated with this conspecific representing part of the context. Williams and his colleagues found that fear and analgesia existed 24 h after a single 15-min resident-intruder defeat session (Williams & Scott, 1989; Williams, Worland, & Smith, 1990). However, these reactions were observed only when intruders were tested in the presence of the alpha-colony odors, as opposed to control odors. Additional evidence that such fear was a result of conditioning was revealed by the fact that extinction trials, in which defeated intruders were reexposed to the alpha-colony odors before testing, reduced fear and analgesia during later test sessions when these odors were present (Williams, Rodgers, & Adler, 1990).

The defeat session and the use of an alpha conspecific's odors are more naturalistic conditioned stimuli than those used in most laboratory studies. It has been claimed that ethologically relevant stimuli should be more related to the rat's natural development of fear (Treit, Pinel, & Fibiger, 1981). The special relationship between defeat and fear can be perceived as having adaptive significance, because the fear of the defeated rat to the odor of the alpha colony would normally result in its avoiding such stimuli in the future (Williams & Groux, 1993). Thus, using ethological stressors as opposed to artificial stressors (e.g., shock) enables one to discover how conditioning processes

can regulate an organism's reaction to stimuli in its natural environment.

Experimentation reveals that the GABA (γ -aminobutyric acid) receptor complex plays a fundamental role in the development of fear. Short and Maier (1991) found that blocking the benzodiazepine receptor site during inescapable shock, by administering an antagonist via a cannula into the dorsal raphe, prevented the occurrence of subsequent fear-induced behavior. Specifically, the blocking of the benzodiazepine receptor was found to have no intrinsic effect, yet it was capable of preventing future fear. The potential for an endogenous inverse agonist to be released by the dorsal raphe is currently thought to be critical in the development of fear (Graeff & Silveira Filho, 1978; Treit, Robinson, Rotzinger, & Pesold, 1993; Waltschek & Raab, 1982).

More pertinent to the present research is the fact that when benzodiazepines bind to the GABA receptor, decreases in neuronal transmission occur and fear can be significantly reduced (Shephard, 1987). If the alterations of the dorsal raphe normally induced by fear are blocked by the administration of a benzodiazepine, the organism should not be able to associate fear with the context. This hypothesis has been supported through the successful blockage of fear conditioning via such benzodiazepines as diazepam and midazolam, and to visual and contextual cues associated with painful laboratory stressors, such as electric shock (Fanselow & Helmstetter, 1988; Westbrook, Greeley, Nabke, & Swinbourne, 1991).

In addition to the GABA receptor system, attention has focused on the serotonin receptor system and fear-mediated reactions (Chopin & Briley, 1987; Traber & Glaser, 1987; Treit, 1991). Increased levels of serotonin lead to an increase in fear (Chopin & Briley, 1987), and exposure to

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stress increases serotonin levels in the rat brain (Adell, Garcia-Marquez, Armario, & Gelpi, 1988). The raphe nuclei, and connections to the septo-hippocampal system, have been found to increase in activity when an animal is exposed to a stressor. This structure also contains high concentrations of serotonergic neurons and hence is considered to be the area where serotonin has its effect on fear (Eison & Temple, 1986).

The 5-HT_{1A} receptor is the predominant serotonin receptor in the hippocampus and in parts of the limbic system as well as the septum and raphe nuclei (Chopin & Briley, 1987; Glaser, Rath, Traber, Zilles, & Schleicher, 1985; Traber & Glasner, 1987). This abundance of the 5-HT_{1A} autoreceptors suggests that agonists at this receptor would be useful anxiolytic agents. Indeed, buspirone, an agonist of the 5-HT_{1A} autoreceptor, decreases serotonergic action and relieves fear (Blanchard, Rodgers, Hendrie, & Hori, 1988; Costall, Kelly, Naylor, & Onaivi, 1988). Furthermore, it has been demonstrated that activation of 5-HT_{1A} autoreceptors can be as effective in reducing fear as are benzodiazepines (Fernandez-Guasti & Hong, 1989; Treit & Fundytus, 1988). Therefore, it is speculated that buspirone might disrupt the conditioning of fear to the context associated with a stressor that would normally activate the serotonin system. Presently, no research findings have been reported that have demonstrated this effect.

EXPERIMENT 1

Pinel and other researchers have shown that the "defensive-burying paradigm" provides several measures of fear during a single testing session (Pinel & Mana, 1989; Pinel & Treit, 1978). Specifically, following a brief (e.g., 100-msec) shock from a wall-mounted prod, rats freeze in a crouched, immobile posture facing the prod. Then, after a series of approach and avoidance responses, they bury the prod with the bedding material by means of a repetitive motion of their forepaws (Moser & Tait, 1983). Both freezing and burying are considered species-specific defensive reactions that occur in response to aversive situations, in both wild and laboratory settings (Fanselow, Sigmundi, & Williams, 1987). Another aspect of the burying paradigm, which is particularly relevant to the present experiment, is that it enables the researcher to introduce odors (e.g., the soiled bedding of the alpha colony) into burying material prior to the shock-prod testing session.

As noted, Williams and Scott (1989) found that a single session of being attacked and defeated by an aggressive alpha conspecific was later found to *suppress* prod burying and increase freezing, provided that alpha-colony odors were present during the test session. The purpose of Experiment 1 was to determine whether benzodiazepine and serotonin manipulations would result in blocking these conditioned reactions to the alpha-colony odors shown by defeated subjects. Specifically, it was hypothesized that both a benzodiazepine (i.e., diazepam)

and a 5-HT_{1A} autoreceptor agonist (i.e., buspirone) would block the *conditioning* of fear in defeated rats and therefore prevent a suppression in prod burying and an increase in freezing during shock-prod tests conducted 24 h after the defeat session.

Method

Subjects

Subjects were 48 experimentally naive male rats, approximately 350 g, bred in the Kenyon College Psychology Department Laboratory from Sprague-Dawley (Harlan) descent. Two rats were maintained in each cage with food and water available ad lib, until 1 week before manipulation, at which time rats were placed in separate cages in the same room. A 12:12-h light:dark schedule was established by a timer. Testing occurred during the light part of the 12-h schedule. Ten colonies of 2 male rats of 2 years and 1 younger female rat were used as aggressive colonies. At least 1 of the male rats in each colony was consistently aggressive, frequently biting nonsubject intruders during preliminary defeat-training sessions. For more details on identifying aggressive residents, see the methodology used by Williams and his colleagues (Williams, 1982; Williams, Just, & Worland, 1994; Williams & Scott, 1989; Williams, Worland, & Smith, 1990).

Apparatus

The rats in the 10 colonies were housed in polypropylene tubs (50 × 40 × 20 cm) with stainless-steel wire tops. A corn-cob bedding material ("Bed-O-Cobs") covered the floor and was changed approximately every 2 weeks, but not before defeat sessions (to ensure proper odor conditioning).

Prod-burying tests, conducted in a separate room from the defeat/nondefeat room and colony rooms, involved placement of the subjects in a metal box (36 × 36 × 41 cm). This box had an opening at the top, to which a ventilated Plexiglas lid was attached. The bottom of the box was covered with an 8-cm layer of the same type of corn-cob bedding used in the defeat/nondefeat colony cages. The respective odors of colonies used during defeat sessions were spread in a 2-cm layer on the bedding immediately prior to testing. On one side of the box, 2 cm above the bedding, was placed a wooden dowel (15 mm in diameter) with two noninsulated copper wires coiled in a parallel fashion. These copper wires were attached to a Lafayette 82400 shock source, which enabled the experimenter to manually administer shock when subjects touched the shock prod with both forepaws. A Panasonic WV-3260 video camera, mounted above the shock-prod test box, enabled the behavior of the subjects to be monitored and recorded in an adjacent room on a Sony SSM-2010 color monitor. A white noise generator was used during defeat sessions and prod-burying tests to produce a background noise of 75 dB SPL, to ensure that distracting sounds did not disturb the animals. This apparatus has been used previously in prod-burying tests (Williams et al., 1990; Williams & Scott, 1989).

Drugs

Drugs used at the time of defeat/nondefeat were as follows: a benzodiazepine receptor agonist, diazepam (DZP), at 2.5 mg/kg; a 5-HT_{1A} autoreceptor agonist, buspirone (BUS), at 5.0 mg/kg; and an equivalent amount of physiological saline (SAL) (the vehicle for the other drugs) as a control. Dosages were based on prior research and pilot studies.

The dosage of diazepam used, 2.5 mg/kg, has been previously found to block freezing conditioning through footshock (Fanselow & Helmstetter, 1988; Experiment 4) without producing noticeable side effects. Further, in pilot studies using 5 mg/kg of diazepam, noticeable motoric side effects were found during defeat sessions, but these side effects were not found at 2.5 mg/kg. Evidence that the 2.5-mg/kg level does not produce disturbing side

effects also comes from the fact that defensive behavior was measured during the defeat sessions and compared to both the buspirone and saline conditions. The known low solubility of diazepam in saline did not prove to be a problem because (1) the dosage was only 2.5 mg, (2) it was prepared immediately prior to use, and (3) the mixture was shaken until a homogeneous solution with no precipitate was observed. The success with saline as a vehicle for diazepam precluded the use of a special vehicle that would differ from that given to the buspirone and control groups.

No research to date has focused on the ability of buspirone to prevent the conditioning of fear. However, the dosage of 5 mg/kg has been shown to be effective in preventing learned helplessness behavior noticed after inescapable shock (Drugan, Crawley, Paul, & Skolnick, 1987) and in reducing reactions associated with anxious states (Blanchard et al., 1988). No side effects were noticed at this dosage during pilot studies. As with diazepam, the measure of behavior during defeat sessions to ensure equal activity at this time provided assurance that no disturbing side effects were present. Finally, any drug-induced disruption in the subject's ability to perform normally during later testing (e.g., burying of the shock prod) would have produced results opposite to the researchers' hypothesis.

Procedure

Subjects were placed in habituation chambers twice daily for 30-min sessions on 2 consecutive days (four sessions total). Following this, six groups of 8 rats were randomly assigned to each of the six cells of a 2×3 factorial design: saline administered at the time of nondefeat (Group SAL/ND); diazepam administered at the time of nondefeat (Group DZP/ND); buspirone administered at the time of nondefeat (Group BUS/ND); saline administered at the time of defeat (Group SAL/D); diazepam administered at the time of defeat (Group DZP/D); and buspirone administered at the time of defeat (Group BUS/D).

Subjects were injected 30 min before the defeat and nondefeat sessions to ensure that the drugs were absorbed. Subjects experiencing defeat received a 25-min defeat session in a 2-male colony, with the female temporarily removed. If the subject displayed freezing behavior, in an upright or down posture, it was scored as defensive behavior during 10-sec intervals on a chart recorder. The number of bites given by the aggressive males was counted to determine if the level of aggression was equal for the groups experiencing resident-intruder sessions. The groups not exposed to defeat sessions were placed in a tub with the odors of the aggressive colonies, but without the colony rats being present.

Twenty-four hours after the defeat and nondefeat sessions, subjects were placed in shock-prod chambers with the respective odors of their prior experience. When subjects placed both forepaws on the shock prod, one shock of 6.5 mA was administered for less than 100 msec. As with Pinel and Treit (1978), after administration of the shock the animal was removed from the chamber and placed in its home cage for 1 min, and then placed back into the shock-prod chamber. This 1-min interval has been shown not to disrupt the association of the prod with the shock, and such a relationship can be found to last for 20 days after shock (Pinel & Treit, 1978). The replacement of the subject back into the shock-prod chamber marked the beginning of a 25-min testing interval. During this time the experimenter measured the cumulative freezing and prod-burying durations from a video monitor in an adjacent room. Freezing was defined as no movement except for respiration. Prod burying was defined as the repetitive motion of the forepaws, moving the bedding material toward the shock prod (Pinel & Treit, 1978). If a subject buried the shock prod, the highest portion of the pile, within a 5-cm radius of the prod, was recorded.

Results and Discussion

Analysis of the behavior during the defeat sessions was conducted to determine if all of the defeated groups

had similar defeat experiences. The results of an analysis of variance (ANOVA) indicated that all of the defeated groups received a comparable number of bites by the aggressive colony rats (overall average of 2.58 bites per session with a range of 1.88 to 3.00), and no differences were significant at the .10 level. A separate ANOVA showed that the defeated groups engaged in similar levels of defensive behavior during the defeat experience (overall average of 119.42 defensive responses with a range of 112.12 to 131.75), which also revealed that there were no group differences at the .10 level. These findings indicate that all the defeated groups were equally attacked and defensive during the resident-intruder sessions. This also implies that the drugs did not result in side effects that might have altered the degree or nature of responses that occurred during the agonistic encounters.

Blind reexamination of videotaped responses scored during the prod-burying tests revealed that there were very high levels of intrarater reliability, resulting in *r* values exceeding .94.

The mean durations of prod burying are depicted in Figure 1. An ANOVA revealed a significant overall main effect of the defeat/nondefeat session on prod burying [$F(1,46) = 3.98, p = .05$]. This confirms other research showing that previous stress exposure, such as defeat,

Figure 1. Mean duration of burying by subjects during 25-min prod-burying tests. Subjects either experienced no defeat session (ND) or one defeat session (D) 24 h prior to testing. Subjects received saline (SAL), diazepam (DZP), or buspirone (BUS) 30 min prior to ND/D sessions.

produces a lack of response in the prod-burying paradigm when prior exposure cues are present (Williams & Scott, 1989). More importantly, planned comparisons showed that defeat groups receiving diazepam or buspirone (Groups DZP/D and BUS/D) buried longer than the control group, SAL/D ($p < .05$). Further comparisons showed that the burying of the DZP/D and BUS/D groups was not significantly different from that of the other nondefeat groups (i.e., Groups SAL/ND, DZP/ND, and BUS/ND) at the .05 level. Therefore, the administration of diazepam or buspirone was sufficient to abolish the lack of prod-burying behavior that was observed for the SAL/D group. Hence, the contingency between a state of fear and the odors of the alpha colony was never established. It is also important to note that burying responses were consistently in the direction of the shock prod, supporting its role as a localized source of fear.

The mean heights of the bedding piles made by the subjects are shown in Figure 2. The results of the pile heights are quite similar to those of the duration of prod burying. An ANOVA revealed a significant main effect of drug type [$F(2,45) = 4.60, p < .05$]. Further, planned comparisons revealed that the DZP/D and BUS/D groups had significantly higher piles than those of the SAL/D group ($p < .01$). As with the mean duration of prod burying, further statistical comparisons showed that the resident-intruder defeated subjects that received diazepam (Group DZP/D) and those that received buspirone (Group BUS/D) did not significantly differ from

Figure 2. Mean height of piles buried by subjects during 25-min prod-burying tests. Subjects either experienced no defeat session (ND) or one defeat session (D) 24 h prior to testing. Subjects received saline (SAL), diazepam (DZP), or buspirone (BUS) 30 min prior to ND/D sessions.

Figure 3. Mean duration of freezing by subjects during 25-min prod-burying tests. Subjects either experienced no defeat session (ND) or one defeat session (D) 24 h prior to testing. Subjects received saline (SAL), diazepam (DZP), or buspirone (BUS) 30 min prior to ND/D sessions.

the other nondefeat groups in terms of pile height. The similar patterns for the mean durations of prod burying and the mean height of piles is important to note because an inconsistency would imply the disruption of a subject's burying ability, which might be due to motoric side effects of the drugs.

The mean durations of freezing for the six groups appear in Figure 3. An ANOVA revealed main effects of freezing, which yielded significant effects of the defeat/nondefeat experience [$F(1,46) = 33.95, p < .001$], drug type [$F(2,45) = 30.55, p < .001$], and the interaction of defeat/nondefeat experience and drug type [$F(1,46) = 27.83, p < .001$]. Planned comparisons showed that the resident-intruder defeat group receiving saline (Group SAL/D) displayed significantly more freezing than all other groups and that the other groups did not significantly differ from each other ($p < .01$). The means of the freezing for the six groups are basically an inverse pattern of the prod-burying results. Such an inverse relationship is expected, since an increase in freezing represents increased fear while an increase in prod burying represents a decrease in fear in the context of the alpha-colony odors.

The three measurements of fear from the prod-burying paradigm used in Experiment 1 yielded results showing that the conditioning of fear to odor cues during defeat sessions can be blocked by administration of either DZP or BUS. It is not likely that these results are due to dis-

turbing side effects of these drugs, since such disruptions would have caused a decrease, rather than an increase, in the responses. Furthermore, diazepam and buspirone were both able to block such conditioning, as evidenced by the fact that neither Group DZP/D nor Group BUS/D was significantly different from the nondefeat group on any measure, nor were these groups ever different from each other.

EXPERIMENT 2

The experience of an aversive event is also involved in an organism's response to pain and the expression of analgesia (i.e., reduction in perception and/or reactivity to pain). Analgesia is an adaptive reaction to pain that prevents behaviors associated with attending to tissue damage (i.e., recuperative behaviors) and that allows an organism to engage in defensive reactions in order to increase chances of survival in a dangerous situation (Fanselow & Baackes, 1982). This implies that defensive reactions as behavioral manifestations of fear and analgesia frequently coexist.

This simultaneous display of fear and analgesia suggests that both can be conditioned to the same stimuli during exposure to a stressor. Support for this proposition comes from a study by Fanselow (1984) showing that when rats were given a few inescapable shocks in one chamber and tested in another, fear and analgesia were observed only when subjects were later tested in the same chamber used during exposure to the stressor. Further evidence that the analgesia was a conditioned reaction is that when the context was presented without painful stimuli before testing, extinction occurred, and fear and analgesia were no longer observed (Fanselow, 1984; Experiment 5).

The concurrent conditioning of analgesia and fear has also been demonstrated in naturalistic studies involving defeat sessions by an aggressive conspecific (Williams, Just, & Worland, 1994; Williams, Worland, & Smith, 1990). As with the conditioning of fear, the conditioning of analgesia during a single session of defeat was found to be dependent on the odor cues during the defeat sessions, so that if the odors were not present during testing, analgesia did not occur (Williams et al., 1990; Experiment 1). Finally, exposure to the alpha-colony odors for an extended time period was found to extinguish the association between the odors and analgesia, providing further evidence that this analgesia is a result of conditioning (Williams et al., 1990; Experiment 3).

The GABA receptor mechanism is involved in the conditioning of analgesia much the same way as it is involved in the conditioning of fear, such that the reduction of conditioned analgesia is possible upon benzodiazepine agonist administration during stressor exposure. Fanselow and Helmstetter (1988) demonstrated that benzodiazepine administration during footshock disrupted the conditioning of fear and analgesia to the experimental context. On the basis of this evidence, they suggested

that fear and analgesia can be mediated, and conditioned, through a common fear-like process.

A more ethological approach to the study of benzodiazepine administration and the blockage of analgesia has been reported by Rodgers and Randall (1987a, 1987b, 1988a, 1988b). These experimenters administered benzodiazepines during resident-intruder sessions and tested for analgesia both before and after such sessions. Although the administration of benzodiazepines attenuated analgesia following the resident-intruder experience, the degree to which this procedure involves *conditioned* analgesia is questionable because the environmental context was not the same during the resident-intruder sessions and the test sessions. Furthermore, the lack of a time interval between the resident-intruder experience and testing complicates the distinction between the ability of benzodiazepines to alter the immediate aftereffects of resident-intruder defeat sessions as a stressor versus the expression of conditioned analgesia during a subsequent test session.

Experimental procedures similar to those involving benzodiazepines have been used with the 5-HT_{1A} system in analgesia (Rodgers & Randall, 1987a; Rodgers & Shepherd, 1989a, 1989b). Again, an increase in analgesia was blocked if 5-HT_{1A} autoreceptor agonists were administered prior to resident-intruder sessions, as compared with a prestressor level of responding to pain. However, as noted, the ability to declare such analgesia as *conditioned* has not been examined.

Experiment 2 was conducted to examine the ability of benzodiazepine and a 5-HT_{1A} autoreceptor agonists to block the conditioning of analgesia. To ensure that the analgesia was a result of a fear process and concurrently occurred with fear, the resident-intruder defeat procedure for this experiment was the same as that in Experiment 1. The degree to which the separate receptor systems are involved in the conditioning of fear and analgesia was assessed using odor manipulations to ensure that similar contextual cues were present during both the defeat sessions and the later test session. On the basis of the results of Experiment 1 and the possibility for fear and analgesia to be mediated by a common fear-like process, it was assumed that diazepam and buspirone would both be effective in blocking the conditioning of analgesia.

The formalin test was used to measure analgesia in this experiment. This test involves the injection of a very dilute solution of formalin to the right dorsal paw, which is painful to nonstressed subjects (Fanselow, 1984). As with other tests of pain sensitivity (e.g., tailflick and hot plate), analgesia is inferred from a lack of response by the subject. For the formalin test, this means that the subject will take longer to lick the injected paw and/or will lick it fewer times than animals not experiencing analgesia. An additional reason this test was chosen is that odor manipulations are easily conducted by the addition of odorants to preexisting bedding (Williams et al., 1994; Williams, Worland, & Smith, 1990). Finally, mea-

sures of freezing were also obtained during testing for analgesia as an indication of conditioned fear.

Method

Subjects

All aspects of the subjects and colonies were identical to those of Experiment 1.

Apparatus

As with Experiment 1, the rats in the 10 colonies were housed in polypropylene tubs ($50 \times 40 \times 20$ cm) with stainless steel wire tops. A corn-cob bedding material covered the floor and was changed approximately every 2 weeks, but not before defeat sessions to ensure sufficient odor conditioning. In a separate room, formalin injections were given to each subject's right hind paw, while confined in a restrainer. Formalin-test observations, in another room, involved placement of the subjects in $50 \times 40 \times 20$ cm polypropylene tubs. The respective odors of colonies used during the defeat and nondefeat sessions were placed in the bedding immediately before formalin testing. A video camera, mounted above the formalin-test observation tub, enabled the behavior of the subjects to be monitored in an adjacent room. All video and white-noise equipment and procedures were the same as those described in Experiment 1.

Drugs

The rationale of drug choices and dosages for this study were identical to those in Experiment 1.

Procedure

The subjects were handled during four sessions, one on each consecutive day. Subsequently, groups of 8 subjects were randomly assigned to each of the six cells of the 2×3 factorial design, which was identical to that of Experiment 1. All aspects of the defeat session were identical to those of Experiment 1.

Twenty-four hours after the defeat and nondefeat sessions, subjects were given a 0.05-ml injection of 15% formalin solution under the dorsal surface of the right rear paw. This concentration has been shown to produce pain and paw licking, but not a "ceiling effect" so that prior manipulations cannot be noticed during formalin testing (Fanselow, 1984; Fanselow & Helmstetter, 1988; Williams, Just, & Worland, 1994; Williams, Worland, & Smith, 1990). After being returned to their home cages for 15-min, all subjects were given a 1-min priming session. Resident-intruder defeated subjects were placed in the same colony in which they had experienced defeat sessions, and the nondefeated subjects were placed in a novel, aggressive colony. The time interval and the methods of this priming procedure have been extensively documented (Jackson, Maier, & Coon, 1979; Williams, Just, & Worland, 1994; Williams, Worland, & Smith, 1990).

As with Williams, Worland, and Smith (1990), after this priming session, subjects were placed in the formalin testing room in a tub with bedding odors from their defeat or nondefeat environment. An 8-sec time-sampling procedure was used for 16-min to record (1) *freezing*—a crouched posture with no movement except for respiration; (2) *paw licking*—grasping and licking the formalin-injected paw while lying down; and (3) *general activity*—all other behaviors. This 8-sec time period has been adopted as a standard for the formalin test by a number of investigators (Fanselow & Helmstetter, 1988; Williams, Just, & Worland, 1994; Williams, Worland, & Smith, 1990).

Results and Discussion

Analysis of the groups that were given defeat sessions was conducted to ensure that all defeat groups had similar defeat experiences. ANOVAs indicated that not only

did each defeat group receive a comparable number of bites by the aggressive colony rats (overall average of 3.92 bites per session with a range of 2.62 to 5.25), but also that defeated groups showed similar levels of defensive behavior during the defeat sessions (overall average of 110.63 with a range of 108.62 to 113.37); neither ANOVA showed significant effects at the .10 level. As with Experiment 1, this demonstrates that all subjects were equally attacked and defensive during the defeat sessions. Therefore, any differences among these groups during later testing cannot be attributed to differential resident-intruder defeat experience.

Upon blind reexamination of videotaped formalin tests, intrareliability of scoring for the number of observations of both freezing and paw licking resulted in $rs > .95$.

The results of the freezing are similar to those of Experiment 1 and are depicted in Figure 4. An ANOVA revealed significant main effects for the number of observations of freezing during the formalin tests due to defeat/nondefeat experience [$F(1,46) = 10.83, p < .01$], drug type [$F(2,45) = 5.93, p < .01$], and the interaction between defeat/nondefeat experience and drug type [$F(2,45) = 6.24, p < .01$]. As expected, the defeated subjects displayed more freezing than did the nondefeated subjects, and those groups receiving DZP or BUS displayed less freezing. Planned comparisons revealed that the SAL/D subjects displayed more freezing than any

Figure 4. Mean number of observations of freezing during formalin tests shown by nondefeated subjects (ND) or subjects receiving one defeat session (D) 24 h prior to testing. Subjects received saline (SAL), diazepam (DZP), or buspirone (BUS) 30 min prior to ND/D sessions.

other group ($p < .01$). Furthermore, there were no significant differences among the remaining groups ($p < .01$). This supports the results of Experiment 1 in that the conditioning of fear to the odor cues, in terms of freezing, was significantly blocked by both DZP and BUS for the defeated subjects (i.e., Groups DZP/D and BUS/D).

Figure 5 displays the means for paw licking of the six groups during formalin tests. An ANOVA of the paw-licking data revealed main effects for defeat/nondefeat experience [$F(1,46) = 6.26, p < .05$] and type of drug administered [$F(2,45) = 3.99, p < .05$], as well as an interaction between defeat/nondefeat experience and drug administered [$F(2,45) = 5.13, p < .01$]. As expected, planned comparisons verified that the SAL/D group displayed less paw licking (i.e., increased analgesia) than both the DZP/D and BUS/D groups and all nondefeat groups ($p < .01$). Further, no differences existed between any of the remaining groups at an alpha level of .05. Hence, DZP and BUS successfully blocked the conditioning of analgesia to the odor cues during defeat sessions.

The findings of Experiment 2 supported the results of the previous experiment in that both DZP and BUS successfully blocked the conditioning of fear. This experiment again demonstrated that both DZP and BUS completely blocked the conditioning of analgesia for resident-intruder defeated subjects (i.e., Groups DZP/D and BUS/D). The results of Experiment 2 also support those of Experiment 1 in that no differences existed among the DZP/D, DZP/ND, BUS/D, BUS/ND, or SAL/ND groups. Thus,

these findings were not due to any intrinsic actions of the drug themselves; rather, they indicate that these agonists clearly blocked fear conditioning. As predicted from past research, fear-elicited freezing and a reduction in pain sensitivity occurred simultaneously when non-drug-defeated subjects were later tested in the presence of alpha-colony odors. Therefore, the findings clearly support the contention that benzodiazepine and 5-HT_{1A} are involved in the conditioning of fear and analgesia, using defeat sessions as a stressor and alpha-colony odors as conditioned stimuli.

GENERAL DISCUSSION

A session of defeat by a conspecific has previously been shown to result in the conditioning of fear and analgesia to the odor cues present at the time of defeat, and these conditioned reactions can be observed 24 h after the defeat experience only when the odors are present (Williams, Just, & Worland, 1994; Williams & Scott, 1989; Williams, Worland, & Smith, 1990). The ability of a benzodiazepine agonist, diazepam, to block the conditioning of fear and analgesia to the naturalistic stressor of defeat sessions is consistent with prior research using shock as a stressor (Fanselow & Helmstetter, 1988; Westbrook et al., 1991). Ample evidence exists for the efficacy of 5-HT_{1A} autoreceptor activation in reducing fear (Blanchard et al., 1988; Costall et al., 1988; Treit et al., 1993). Likewise, the ability of both the benzodiazepines and the 5-HT_{1A} agonists to reduce fear associated with a shock prod has also been shown (Fernandez-Guasti & Hong, 1989; Treit & Fundytus, 1988). However, the present experiments are the first to demonstrate the ability of these dosages of the benzodiazepine, diazepam (2.5 mg/kg), and the 5-HT_{1A} autoreceptor agonist, buspirone (5.0 mg/kg), to block the conditioning of fear and analgesia resulting from the naturalistic stressor of defeat. The 24-h interval between stressor exposure and testing as well as the use of odors as the contextual stimuli during both exposure and testing ensure that the fear and analgesia displayed in these experiments were, in fact, conditioned reactions (Williams, Just, & Worland, 1994; Williams & Scott, 1989; Williams, Worland, & Smith, 1990).

The simultaneous occurrence and conditioning of fear and analgesia is considered to be advantageous to the animal's future existence. More specifically, the existence of a fear state elicits species-specific defensive reactions, and analgesia permits these reactions to be carried out rather than the animal engaging in recuperative behavior, such as paw licking (Fanselow, 1984). In fact, Fanselow stated that painful stimulation is neither necessary nor sufficient for analgesia to occur, since fear is an integral aspect of this response. Further, it is the expectancy of a dangerous or painful stimulus, rather than the stimulation itself, which elicits concurrent fear and analgesia. Therefore, disruption of an association, or the ability of an association to be made, between an aversive stimulus and a state of fear would suffice to prevent the future occurrence of conditioned fear and analgesia. In

Figure 5. Mean number of observations of paw licking during formalin tests shown by nondefeated subjects (ND) or subjects receiving one defeat session (D) 24 h prior to testing. Subjects received saline (SAL), diazepam (DZP), or buspirone (BUS) 30 min prior to ND/D sessions.

the present experiments, this conditioning process was successfully disrupted by administration of diazepam or buspirone prior to the defeat session. This explains why the defeated subjects that received diazepam or buspirone responded in an equivalent manner as nondefeated subjects during testing, yet demonstrated defensive behavior during the defeat session that was equivalent to the defeated saline-control subjects.

An argument could be that the present results are due to an interference caused by state-dependent learning, as opposed to the blocking of conditioned fear. Such an argument is viable because control groups having saline, buspirone, or diazepam at the time of testing were not employed. Therefore, the state of fear/analgesia could be perceived as having been associated with the state of drug administration, which was not at the time of testing. However, Fanselow and Helmstetter (1988, Experiment 2) employed a training-by-testing counterbalanced design demonstrating that the blockage of conditioned fear and analgesia was not due to state-dependent learning with the use of benzodiazepines. Similar counterbalancing was undertaken to determine if the NMDA antagonist effect of reducing future fear was due to state-dependent learning, and it was revealed that it was conditioning rather than state dependency (Kim, DeCola, Landeira-Fernandez, & Fanselow, 1991). The effectiveness of benzodiazepines in preventing the learned-helplessness escape deficit has also been shown to be void of state-dependent learning effects (Drugan, Ryan, Minor, & Maier, 1984). At present, no prior research has shown the possibility of 5-HT_{1A} agonists to have state-dependent learning effects.

The fact that animals receiving anxiolytics during defeat sessions failed to display conditioned fear and analgesia during testing might lead one to claim that these animals responded to, and hence were treated by, alpha residents differently and thus should not be expected to display the same conditioned fear reactions as the non-drug subjects. Such an argument would not be surprising considering that chronic administration of diazepam and acute administration of the benzodiazepine partial agonist ZK 91296 have been shown to affect both intruder and resident behavior in Wistar rats (Piret, Depaulis, & Vergnes, 1991). However, this effect does not exist with acute administration of diazepam, and the effect of the partial agonist ZK 91296 disappears when expressed as a proportion of the duration of attacks of the residents (Piret et al., 1991). In the present experiments, the number of bites given by the resident, along with the defensive behaviors of the intruder, did not differ among the different conditions of drug/vehicle administration. This lack of differential defeat experiences in these experiments is thought to be a result of the acute administration of the particular drugs and doses chosen, the extreme aggressiveness of our alpha residents, and/or the use of Sprague-Dawley rats

A limitation of the present experiments is the lack of dose-response data. Such an absence could allow for incorrect assumptions to be made regarding the compara-

ble effectiveness of drugs, as each single dosage is only a glimpse of the drugs' effects. However, our dosages were carefully chosen on the basis of previous research involving stressor exposure (Blanchard et al., 1988; Drugan et al., 1987; Fanselow & Helmstetter, 1988). Furthermore, the findings of pilot studies and the analysis of behavior during the defeat sessions, after drug administration, enabled specific dosages to be selected that reduced conditioning but did not impair the motor/motivational aspects of the subjects. Therefore, the absence of dose-response data does not affect the credibility of these results, although more information regarding the comparable effectiveness of these drugs could be ascertained by conducting a dose-response study.

The present experiments determined that the chosen dosages of both diazepam and buspirone were effective in blocking the conditioning of fear and analgesia associated with the stressor of defeat by a conspecific male. The ability of both compounds to have such an effect is not surprising considering that both the benzodiazepine and 5-HT_{1A} receptors have been shown to reduce fear reactions to artificial aversive stimuli, such as shock. Furthermore, both types of these compounds are known to influence the dorsal raphe and alter transmission to the amygdala (Parent, Descarries, & Beaudet, 1981; Taylor, Eison, Riblet, & Vandermaelen, 1985; Traber & Glasner, 1987). However, more research is necessary to determine the role of the dorsal raphe and the amygdala in terms of their pharmacology and the regulation of conditioned fear and analgesia, particularly when organisms have been exposed to stressors found in their natural environment.

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