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Alcohol, anxiolytics and social stress in rats

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Abstract The main objective was to compare the anxiolytic-like profiles of alcohol, diazepam and gepirone along the stress intensity gradient which characterizes consecutive phases of a social confrontation. The acute social stress situation consisted of initially placing the experimental rat as an intruder into the homecage of a resident while the resident was not present, termed the "anticipatory" phase, thereafter permitting brief physical agonistic interactions with the re-introduced resident until the intruder was forced into a submissive supine posture and emitted ultrasonic vocalizations (USV), and eventually exposing the intruder to the resident's threats for 1 h, while being shielded from potential injurious attacks. The hyperthermia, measured via telemetry, in the "anticipatory" phase prior to defeat and in reaction to threats, was decreased by alcohol, gepirone and diazepam; alcohol and gepirone were also effective in attenuating "anticipatory" tachycardia. Alcohol, like gepirone and diazepam, also decreased defensive responses and ultrasonic vocalizations in the "anticipatory" phase of the confrontation, but none of these drugs affected defensive reactions to threats which immediately followed defeat. Gepirone had no systematic sedative effects throughout the confrontation; infact, it dose-dependently reduced the stress-induced suppression of locomotor activity during the "anticipatory" phase. In contrast, at higher doses, alcohol as well as diazepam had marked sedative effects as evidenced by several behavioral parameters (i.e. lie, crouch, walk). The anxiolytic-like profile of hyperthermia, tachycardia, USV and defensive behavior in the "anticipatory" phase of the confrontation by alcohol, gepirone and diazepam contrasted with the lack thereof during the more intense reactive phase. This differential pattern of effects appears to be relevant to the clinical distinctions between anticipatory anxiety and other affective disturbances.

Key words Stress · Agonistic behavior · Telemetry · Autonomic · Core temperature · Cardiovascular · Defensive behavior · Ultrasonic vocalization · rats · Anxiolytics · Alcohol · Diazepam · Gepirone · 5-HT · Benzodiazepines · Social stress · Distress calls · Aggression

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Introduction

Alcohol and benzodiazepines share anti-anxiety effects in humans and potentiate each other (Hommer et al. 1987; Ticku et al. 1992). Most experimental protocols or "models" in animals for predicting anxiolytic activity detect the attenuation of stress-induced behavioral, physiological and endocrine effects by benzodiazepines and ethanol (Lal and Emmett-Oglesby 1983; Aston-Jones et al. 1984; Insel et al. 1984; Koob and Thatcher-Britton 1985; Becker and Hale 1991). Alcohol and benzodiazepines restore responding suppressed by punishment (Barrett et al. 1985; Koob et al. 1986), whether the behavior is conditioned or unconditioned.

More recently developed compounds, which include the azapirones, buspirone, ipsapirone and gepirone, that act directly on the 5-HT_{1A} receptor, begin to show clinical promise in the treatment of generalized anxiety (Browne and Shaw 1991; Taylor and Moon 1991; Wilkinson and Dourish 1991). However, upon acute administration these 5-HT_{1A}-anxiolytics engender variable results in many of the preclinical tests for anxiolytics which were mainly developed and validated for the benzodiazepines (for reviews, see Chopin and Briley 1987; Green 1991; Sanger et al. 1991). In addition to their anxiolytic activity, compounds which act at the benzodiazepine/$GABA_A$ chloride ionophore complex are also sedative, anticonvulsant, muscle relaxant and

amnesic, which may confound traditional tests for predicting anxiolytic effects.

The current experiments employ a strategy for the study of anxiolytic drugs that focuses on an animal's behavior and physiology during social confrontations. This ethological approach comprises an evaluation of several stress-induced responses. Recently, the effects of clinically established and putative anxiolytics have been assessed on behavioral and physiological responses in rats during challenging situations, such as aggressive, predatory, or social confrontations (File 1985; Blanchard et al. 1990; Miczek et al. 1992; Weerts et al. 1993).

Ethologically valid stressors produce immediate and large tachycardia and hyperthermia (Adams et al. 1968; Hajos and Engberg 1986; Fokkema et al. 1988; Borsini et al. 1989; Miczek et al. 1991a; Tornatzky and Miczek 1994). Prolonged social confrontations have been found to lead to essential hypertension in mice and to compromise the chronobiology in the long-term, sometimes with fatal consequences, such as in tree shrews (Von Holst 1985; Eisermann 1992; Henry et al. 1993; Tornatzky and Miczek 1993; Harper et al. 1995).

The social confrontations employed in more recent studies consisted of phases with distinctive intensity gradients of the challenging stimulus (Tornatzky and Miczek 1994; Meehan et al. 1995). In the initial phase, the intruder is exposed to the resident's cage only; in a subsequent phase the intruder reacts to the resident's attack, and finally, the intruder is protected by a wire mesh, but still reacts to the resident's threats. Behavioral and physiological changes are prominent during a period that precedes the actual presentation of salient events such as: handling during measurement of rectal temperature, restraint, pain, food, water, and sexual behavior (Adams et al. 1968; Blackburn et al. 1987; Poncelet et al. 1987; Borsini et al. 1989; Sumova and Jakoubek 1989; Pfaus and Phillips 1991; Mistlberger 1992). In these studies the terms "preparatory" or "anticipatory" were used to describe the conditioned responses in those phases of the experiments which preceded the "reactive" phase in a close temporal proximity (Estes and Skinner 1941). Similarly, intruder rats which have been previously defeated on several occasions already react with an increase in core temperature when exposed to the olfactory cues of the home cage of the potential attacker (Tornatzky and Miczek 1994). This conditioned or "anticipatory" hyperthermia developed in the course of the first three confrontations and is paralleled by a decrease in exploratory and motor behavior and by an increase in defensive behaviors and in two types of ultrasonic vocalizations (USV) emitted in the low (20–30 kHz) and the high (31–70 kHz) frequency range.

Under specific conditions, vocalizations may communicate intense affect (e.g., Kalin and Shelton 1989; Miczek et al. 1991b; Winslow and Insel 1991) and are differentially sensitive to various types of anxiolytic drugs. Rats emit ultrasonic vocalizations (USV) in the low- or in the high-frequency range depending on the behavioral context (Kaltwasser 1990; White et al. 1990; Blanchard RJ et al. 1991; Miczek et al. 1991b; Van der Poel and Miczek 1991; Haney and Miczek 1993; Miczek and Vivian 1993; Vivian and Miczek 1993). The present protocol focuses on animals which exhibit a sensitized level of high-frequency USV and defensive-submissive behavior in conjunction with suppressed exploratory behavior (Tornatzky and Miczek 1994). In the repeatedly defeated intruder, a drug-induced reduction or reversal of suppressed exploratory and locomotory behavior in combination with a concurrent decrease in defensive behavior, USV, tachycardia and hyperthermia may be interpreted as an anxiolytic drug effect.

In the present experiments, ethanol was compared to the prototypic benzodiazepine anxiolytic diazepam and the 5-HT_{1A} agonist gepirone in their effects on autonomic, behavioral and vocal responses in socially stressed intruder rats. The main objective was to characterize the anxiolytic-like profiles of these compounds along the stress intensity gradient which characterizes the consecutive phases of the presently used protocol and to compare them to the profiles of metoprolol and clonidine (Tornatzky and Miczek 1994).

Methods

Animals

Male hooded Long-Evans rats (initially weighing 300–350 g; Charles River, Wilmington, Mass.) were housed individually in standard polycarbonate cages ($22 \times 20 \times 46$ cm^3) placed in a sound-attenuating and ventilated enclosure ($34 \times 30 \times 56$ cm^3). An additional male "resident" rat (400–600 g) which consistently displayed aggressive behavior was pair-housed with a female in a $45.7 \times 45.7 \times 71.1$ cm^3 stainless steel cage, with sawdust covered floor. All animals were maintained with free access to Purina rodent chow and to water in an environmentally controlled vivarium (23 ± 1°C, 40–50% humidity, inverted 12-h light/12-h dark photocycle, lights off at 0800 hours). All animals were cared for in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, NRC. The Tufts Committee on Laboratory Animal Care and Use supervises all procedures.

Apparatus and procedures

The heart rate- (HR) and core temperature- (T_c) dependent radio frequency of the biotelemetry sender (TA11CTA-F40, size: 8 g, 3 cm^3; Mini-Mitter, Sunriver, Ore.) implanted in the peritoneum of each intruder was received by an antenna/receiver board (CTR86) placed under each animal's cage. The computer-based data acquisition system (Dataquest III; Data Sciences, St. Paul, Minnesota) received the raw telemetry data from the antenna boards via a consolidation matrix (BCM-100). The system converted the raw telemetered data into common units (e.g., °C) and was set up to record concurrently HR and T_c from four experimental animals every 5 min on a 24-h basis.

Implantation

The animals were anesthetized with ketamine (15 mg/100 g body wt, IP) and xylazine (0.4 mg/100 g body wt, IM). Using antiseptic procedures, the sterilized senders were inserted through a 1.5 cm incision in the peritoneum and sutured to the abdominal wall. The EKG-sensing shielded leads extended subcutaneously to the right axilla and to the left lower rib area where their unshielded 0.5 cm long tips were sutured to the muscle tissue. The rats fully recovered from anesthesia 1–2 h after the end of surgery and were injected on the following 5 days with antibiotic (Penicillin G, 30 000 IU/day, IM). A 9–12 day postoperative period was allowed for complete recovery.

Social confrontation

Every social confrontation was scheduled at 0900 hours (i.e. 1 h after onset of the dark phase). It began, as described in detail previously (Tornatzky and Miczek 1994), by placing the intruder into the resident's homecage without the resident being present (i.e. "anticipatory phase"). After 10 min the male resident was re-introduced (i.e. "attack encounter") until the unambiguous display of submissive signals by the intruder was observed. The attack encounter usually lasted for 120 s. Immediately after these signs, which included ultrasonic vocalizations and supine posture displayed for 4 uninterrupted seconds, the intruder was placed in a wire-mesh protective cage ($17.8 \times 17.8 \times 33.0$ cm^3) for the following 55 min of the "threat encounter". The protective cage allowed unrestricted auditory, olfactory, and visual contact with the resident but protected the intruder from any potential injury.

Telemetered HR and T_c were sampled every 20 s throughout the social confrontation. During the last 3 min of the "anticipatory" phase of the encounter, during the attack encounter and during the first and the last 5 min of the threat encounter video and audio recordings were performed as described in detail previously (Tornatzky and Miczek 1993, 1994; Vivian and Miczek 1993). Behavior was recorded with an infrared-sensitive video system while ultrasonic vocalizations were recorded on a instrumentation tape recorder in conjunction with a condenser microphone, preamplifier and amplifier (Bruel & Kjaer Type 4135, 2633, 2610, respectively) which provided a flat frequency response in the 20 to 65 kHz range.

Protocols

The social confrontations with a resident, included the "anticipatory" phase, "attack" and "threat encounters". Three separate groups of intruders, which had been defeated a minimum of four times previously were treated prior to social confrontations with diazepam ($n = 8$; 1.0, 3.0, 6.0, 10.0 mg/kg, IP), ethanol ($n = 10$; 0.1, 0.3, 1.0, 1.7, 3.0 g/kg, PO) or gepirone ($n = 9$; 0.3, 0.6, 1.0, 3.0, 6.0 mg/kg, IP). Each rat received all doses of one of the three drugs in a random counter-balanced sequence in weekly intervals; before a second weekly confrontation with the resident, intruders were treated with the drug-appropriate vehicle in order to monitor possible changes that may occur over the course of the weeks of testing. Consecutive confrontations were separated by at least 2–4 days in order to avoid long-term stress effects on circadian rhythmicity. In fact, HR and T_C baselines as well as the difference in these autonomic measures between the light and dark portions of the circadian cycle showed neither systematic changes across consecutive vehicle trials nor dose effects, confirming previous results (Tornatzky and Miczek 1994). In each of the experimental series, behavioral data of two additional intruder rats that were implanted with blood pressure senders were included in the analysis. This increased the data base for behavioral and vocal responses, since they occur much less frequently than physiological signals.

Drugs

Diazepam (Hoffman-LaRoche; 1.0, 3.0, 6.0, 10,0 mg/kg; suspended in a solution containing distilled water, 14% propylene glycol and 1% Tween 80) and gepirone (Bristol-Myers; 0.3, 0.6, 1.0, 3.0, 6.0 mg/kg; dissolved in saline) were administered 20 min prior to attack encounters. Ethanol (US Industrial Chemical; 0.1, 0.3, 1.0, 1.7, 3.0 g/kg; diluted in distilled water in concentrations from 3% to 10% w/v) was administered PO by stainless steel gavage 20 min before attack encounters. Gepirone, diazepam or the appropriate vehicle solutions were administered in a volume of 1 ml/kg body weight. Ethanol solutions were administered in a volume of 10 ml/kg, with the exception of 3.0 g/kg ethanol, which was given in 20 ml/kg.

Data analysis

Behavior

Video records were analyzed by two experienced observers who were blind to the treatment conditions. Behavioral events were observed on a monitor (NEC Model PM1971A) and summarized with a custom-designed PC-based data acquisition system and software. Each observer focused on either the intruder or the resident animal recording the sequence, frequency and duration of walking, rearing, grooming, pursuits, sideways threats, attacks, aggressive postures, nasal and anogenital contact, allogrooming, crouching, lying, feeding and drinking, escapes, supine, and defensive upright postures (Grant and MacKintosh 1963; Miczek 1979). During the threat encounter, every striking movement as well as every digging approach of the resident towards the wiremesh cage was recorded as a "cage attack". The data acquisition system has been described in detail (Miczek 1982; Tornatzky and Miczek 1993). Briefly, each observer recorded behaviors in real-time by depressing one of 16 possible keys on a portable console for the duration of the relevant behavior. The reliability between observers in recording frequency and duration of all acts and postures exceeded 90%.

Ultrasonic vocalization

Analog signals from the instrumentation recorder were analyzed by playback of the audio tape at quarter speed through an amplifier into headphones (Radio Shack Model 33-2002). Trained listeners depressed keys when listening in response to the calls and concurrently viewing the signals on a spectrum analyzer (Tectronix Model 5L4N), in order to distinguish the low (20–30 kHz) or high (31–70 kHz) frequency of USV. The rate of both low- and high-frequency USV and the total duration of the low calls for every recording period were calculated based on the onset and offset times of USV.

Heart rate and core temperature

An individual's baseline was defined as the average of HR or T_c values recorded in its homecage during the dark portion of the light cycle (1200–1800 hours) preceding the test day. During each period before, during and after the social confrontation the difference from baseline was calculated for the intruder's HR and T_c. Preceding the test day the HR and T_c during the light portion of the circadian cycle were recorded from 0000 to 0600 hours in the homecage of the intruder. The average of the values during the first 2 min of the exposure to the resident's home cage prior to defeat, the average of the HR and T_c responses during the 10 min following the beginning of the attack encounter, and the averaged values during the

last 30 min of the threat encounter were used to determine the changes in HR and T_c during the social confrontation. One hour after the intruder was returned into his home cage the average of HR and T_c values recorded during the next 2 h were used to evaluate the intruder's recovery after the social confrontation.

Statistical analysis

ANOVAs for repeated measures were used in order to evaluate the drug effects on autonomic, behavioral or vocal responses during the social confrontations. Comparisons between the mean of vehicle control values and those of different drug doses were tested for significance by contrast procedures, using $P < 0.05$ as criterion after an overall statistical significance for a treatment effect had been found (Wilkinson 1987). All measures of variance in the data refer to standard error of the mean (SEM).

Results

No systematic variation across doses of any of the drugs was detected for the attack behavior of the resident stimulus animal at the beginning and at the end of the 1 h long threat encounter. The HR and T_c baseline data and the attack behavior of the resident rat during the threat encounter are summarized in Table 1 for diazepam, alcohol, gepirone or the appropriate vehicle treatments. It is evident that the autonomic changes of the intruder during and after the social confrontation with the resident relied on stable baseline values. These baselines recorded during the dark phase of the light cycle on the day preceding the social confrontation as well as the lower HR and T_c levels during the light portion of the cycle prior to these confrontations did not change across drugs or doses.

Autonomic changes

Throughout the social confrontation defeat-experienced intruders showed substantially increased heart rate and core temperature which followed an orderly timecourse as portrayed in Fig. 1. In vehicle treated intruders, hyperthermia and tachycardia, which were already present prior to defeat further increased during the attack encounter, remained elevated during the threat encounter and recovered to baseline values approximately 2 h after the rats were returned to their own homecages.

Gepirone effectively attenuated the hyperthermia as well as the tachycardia at the beginning of the 10-min period prior to defeat when the intruder was confronted only with the olfactory cues in the homecage of the potential attacker [see Fig. 5; $F(5, 40) = 30.8$, $P < 0.0001$; $F(5, 40) = 9.9$, $P < 0.0001$]. The 3.0 and 6.0 mg/kg dose caused hypothermia prior to defeat while the hyperthermia during the threat encounter was attenuated [see Fig. 2; $F(5, 40) = 12.5$, $P < 0.0001$]. During the attack and threat encounter gepirone did not systematically change the tachycardic responses (data not shown).

Diazepam, in the dose-range of 3.0–10.0 mg/kg, significantly attenuated the hyperthermia prior to defeat, during the attack and threat encounter [see

Table 1 Heart-rate (*HR*) and core temperature (T_C) baselines of the intruders recorded on the day preceding the social confrontations and the lack of an dose effect of either gepirone, diazepam and ethanol on attack behavior of the aggressive opponent. The baselines and lower values during the light phase of the light cycle are each based on 4 h long telemetered HR or T_c recordings. Attacks by the resident towards the intruder at the beginning ($Threat_5$) and the end ($Threat_{55}$) of the threat encounter are expressed in frequency per minute. All measures refer to means ± SEM

	Period	Dose					
Gepirone (mg/kg)		Vehicle	0.3	0.6	1.0	3.0	6.0
HR ($n = 9$)	Baseline	371.7 ± 6.1	374.6 ± 3.4	364.8 ± 4.8	373.9 ± 7.0	377.9 ± 8.2	371.0 ± 9.1
	Light phase	−70.4 ± 3.8	−78.2 ± 4.4	−71.6 ± 3.3	−68.8 ± 4.9	−71.1 ± 4.1	−70.6 ± 5.6
T_c ($n = 9$)	Baseline	38.0 ± 0.08	38.0 ± 0.09	38.1 ± 0.12	38.0 ± 0.08	38.0 ± 0.09	37.9 ± 0.09
	Light phase	−0.7 ± 0.03	−0.7 ± 0.04	−0.8 ± 0.07	−0.7 ± 0.04	−0.7 ± 0.05	−0.6 ± 0.06
Attack ($n = 11$)	$Threat_5$	6.0 ± 0.6	5.3 ± 0.9	6.4 ± 1.1	5.1 ± 1.0	5.3 ± 0.8	6.4 ± 1.1
	$Threat_{55}$	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.2
Diazepam (mg/kg)		Vehicle		1.0	3.0	6.0	10.0
HR ($n = 8$)	Baseline	363.6 ± 9.8		361.2 ± 8.1	364.3 ± 8.7	365.1 ± 8.0	347.8 ± 9.4
	Light phase	−55.7 ± 6.0		−46.4 ± 7.7	−54.7 ± 5.7	−54.2 ± 3.8	−46.3 ± 4.1
T_c ($n = 8$)	Baseline	37.8 ± 0.1		37.7 ± 0.1	37.8 ± 0.1	37.7 ± 0.1	37.8 ± 0.1
	Light phase	−0.8 ± 0.1		−0.7 ± 0.9	−0.8 ± 0.1	−0.7 ± 0.1	−0.7 ± 0.1
Attack ($n = 10$)	$Threat_5$	7.7 ± 0.8		6.8 ± 1.3	7.4 ± 1.3	5.3 ± 0.9	5.7 ± 0.8
	$Threat_{55}$	0.8 ± 0.3		1.6 ± 0.7	1.1 ± 0.5	0.4 ± 0.2	0.6 ± 0.3
Ethanol (g/kg)		Vehicle	0.1	0.3	1.0	1.7	3.0
HR ($n = 10$)	Baseline	366.9 ± 7.9	368.3 ± 6.8	367.7 ± 6.1	367.2 ± 8.3	368.3 ± 9.2	364.4 ± 7.9
	Light phase	−67.1 ± 4.2	−73.6 ± 8.5	−69.8 ± 4.8	−67.7 ± 4.7	−65.9 ± 6.4	−64.8 ± 3.8
T_c ($n = 10$)	Baseline	38.0 ± 0.07	38.0 ± 0.07	38.0 ± 0.10	38.0 ± 0.09	38.0 ± 0.08	38.0 ± 0.08
	Light phase	−0.8 ± 0.05	−0.8 ± 0.06	−0.8 ± 0.05	−0.8 ± 0.05	−0.7 ± 0.15	−0.8 ± 0.06
Attack ($n = 12$)	$Threat_5$	5.6 ± 1.0	5.0 ± 0.8	5.5 ± 1.2	6.0 ± 1.0	5.8 ± 1.2	7.5 ± 1.0
	$Threat_{55}$	0.5 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.8 ± 0.3	0.6 ± 0.2	0.6 ± 0.3

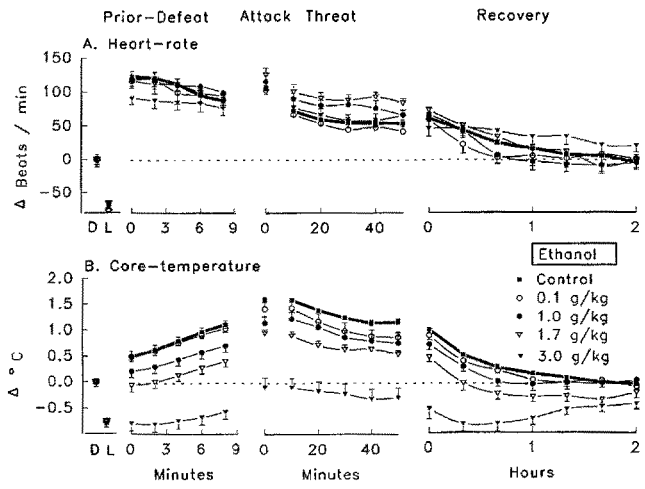

Fig. 1 Ethanol's effects on temporal changes in **A** heart-rate and **B** core temperature reactions of a defeat experienced intruder rat during and after the social confrontation with a resident rat. HR and T_C are given as deviations from the subject's baseline means recorded on the previous day during the dark portion of the light cycle (*D*). Reactions of the ten rats during the light portion of the cycle (*L*) and during the 10 min after begin of the attack encounter are based on the means during these periods, while their reactions recorded prior to defeat, during the threat encounter and during recovery in their homecage are portrayed for consecutive 2-, 10-, and 20 min intervals, respectively. Data are presented as means and SEM

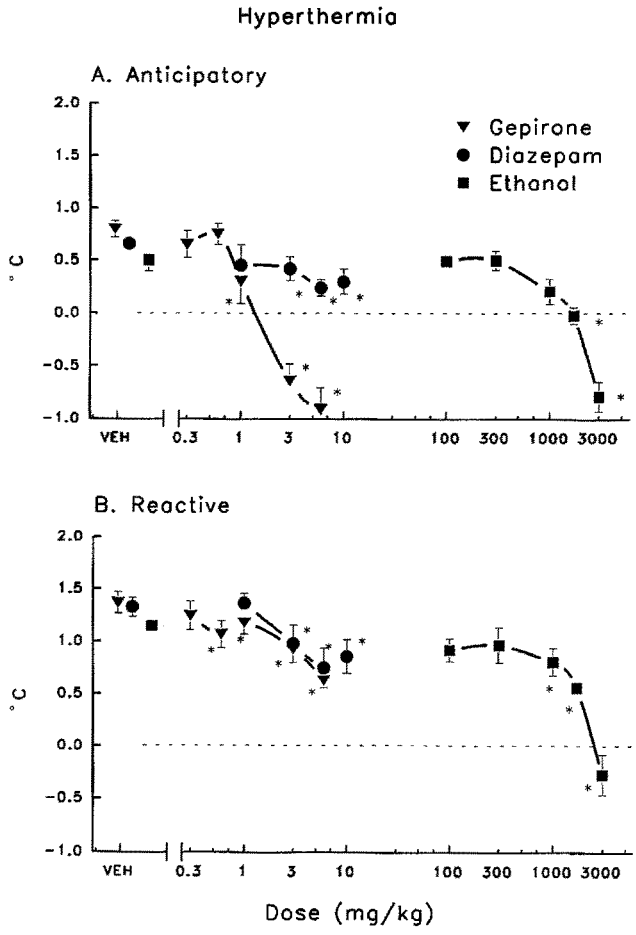

Fig. 2A, B Comparison of the effects of gepirone ($n = 9$), diazepam ($n = 8$) and ethanol ($n = 10$) on stress induced hyperthermia of defeat experienced intruder rats **A** prior to defeat and **B** during the threat encounter. Means and SEM are given and *asterisks* mark mean values which are statistically different from the corresponding vehicle treatment ($P < 0.05$)

Fig. 2; $F(4, 28) = 3.9$, $P = 0.01$; $F(4, 28) = 1.6$, $P < 0.0001$; $F(4, 28) = 0.6$, $P < 0.001$]. The tachycardic response during these periods was not affected by diazepam (data not shown).

Ethanol's pronounced hypothermic effect was significant throughout the social confrontation with the 3.0 g/kg dose decreasing T_C below baseline [for time-course see Fig. 1; prior to defeat: $F(5, 45) = 41.0$, $P < 0.0001$; during attack encounter: $F(5, 45) = 23.9$, $P < 0.0001$; during threat encounter: $F(5, 45) = 21.2$, $P < 0.0001$]. By contrast, tachycardia was decreased by ethanol prior to defeat but was further increased at the 1.0 and 1.7 g/kg dose during the threat encounter [see Fig. 5; $F(5, 45) = 3.5$, $P < 0.01$; $F(5, 45) = 2.8$, $P = 0.03$]. The hypothermia induced by the 1.7 and 3.0 g/kg dose persisted throughout the 3-h recovery period and was accompanied by a significant increase in HR at the highest ethanol dose [see Figs. 1 and 5; $F(5, 45) = 10.7$, $P < 0.0001$; $F(5, 45) = 2.9$, $P < 0.03$]. Neither gepirone nor diazepam systematically affected the HR during recovery from the social confrontation (data not shown).

Behavioral changes

As detailed in Table 2, The most prominent effect was the dose-dependent decrease in defensive upright posture after gepirone, diazepam and alcohol treatment in the "anticipatory" phase [$F(5, 50) = 3.3$, $P < 0.02$; $F(4, 36) = 2.9$, $P = 0.03$; $F(5, 55) = 3.3$, $P = 0.01$]. By contrast, none of the drugs affected this behavior in the period immediately following the defeat (see Fig. 3). At the end of the threat encounter, when the attack behavior of the resident was 10 times less frequent compared to the beginning (see Table 1), the defensive upright posture was again decreased by gepirone, diazepam and ethanol [$F(5, 50) = 2.8$, $P < 0.03$; $F(4, 36) = 2.6$, $P = 0.06$; $F(5, 55) = 2.5$, $P = 0.04$].

Gepirone differed considerably from diazepam and ethanol by having no systematic effects on the duration of the elements crouch and lie throughout the confrontation. In the "anticipatory" phase of the confrontation, gepirone-treated intruders walked more than saline treated controls [$F(5, 50) = 4.7$, $P < 0.01$]. By contrast, diazepam decreased walking prior to defeat, at the beginning and at the end of the threat encounter [$F(4, 36) = 3.0$, $P < 0.03$; $F(4, 36) = 6.5$, $P < 0.01$; $F(4, 36) = 3.0$, $P < 0.03$], while it increased lying prior to [$F(4, 36) = 6.5$, $P < 0.01$] and crouch duration immediately following defeat [$F(4, 36) = 7.5$, $P < 0.002$]. The main sedative effect of the 1.7 and

Table 2 Effects of gepirone, diazepam and ethanol on the intruder's behavioral profile during the social confrontation. The mean ± SEM of the durations of the behavior and the calling rate of 31- to 70 kHz ultrasonic vocalization ("high" USV) are expressed per minute for the following periods of the confrontation: prior to defeat (*Pre*), the beginning ($Threat_5$) and the end ($Threat_{55}$) of the threat encounter. **Bold** type represents $P < 0.05$ for ANOVA and significant contrasts between the measures of the specific drug dose and the corresponding vehicle treatment

	Period	Dose					
Gepirone (mg/kg); n=11		Vehicle	0.3	0.6	1.0	3.0	6.0
"high" USV	Pre	17.3 ± 8.5	16.6 ± 10.1	27.3 ± 11.9	23.1 ± 14.1	16.6 ± 10.7	5.3 ± 2.1
	$Threat_5$	40.8 ± 12.3	57.2 ± 18.1	56.8 ± 17.4	41.2 ± 18.0	24.8 ± 10.8	19.0 ± 7.7
	$Threat_{55}$	22.0 ± 6.1	26.2 ± 9.0	33.4 ± 12.0	19.8 ± 8.4	10.2 ± 6.0	21.2 ± 8.4
Defensive upright	Pre	17.2 ± 4.6	21.6 ± 8.4	12.3 ± 6.1	13.5 ± 5.5	**2.6 ± 1.0**	**0.5 ± 0.4**
	$Threat_5$	20.3 ± 3.8	17.3 ± 3.6	18.0 ± 4.2	19.7 ± 4.6	23.0 ± 4.9	20.5 ± 4.7
	$Threat_{55}$	12.1 ± 2.6	11.1 ± 3.2	14.4 ± 3.6	14.8 ± 5.1	14.5 ± 5.2	**3.9 ± 1.2**
Walk	Pre	4.4 ± 1.5	4.5 ± 2.0	4.0 ± 1.6	3.9 ± 1.7	**8.9 ± 2.2**	**8.8 ± 1.4**
	$Threat_5$	5.2 ± 1.0	5.4 ± 1.0	6.1 ± 1.1	4.7 ± 1.0	3.2 ± 0.9	4.1 ± 0.9
	$Threat_{55}$	2.6 ± 0.6	3.8 ± 0.7	2.9 ± 0.7	2.2 ± 0.7	1.7 ± 0.7	2.2 ± 0.7
Crouch	Pre	30.9 ± 4.0	30.5 ± 7.7	40.8 ± 5.9	37.5 ± 5.9	44.3 ± 2.6	45.9 ± 1.9
	$Threat_5$	28.9 ± 3.2	31.9 ± 2.9	32.1 ± 3.6	32.1 ± 4.0	30.8 ± 4.5	33.8 ± 4.1
	$Threat_{55}$	31.5 ± 3.0	35.0 ± 4.3	29.1 ± 3.8	33.7 ± 5.3	31.5 ± 6.1	33.2 ± 5.5
Lie	Pre	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.7	0.7 ± 0.5
	$Threat_5$	0.1 ± 0.0	1.0 ± 0.7	1.1 ± 0.8	0.5 ± 0.3	1.0 ± 0.7	0.5 ± 0.2
	$Threat_{55}$	8.1 ± 2.0	5.8 ± 2.1	8.2 ± 2.9	7.4 ± 3.7	9.0 ± 4.7	14.3 ± 6.5
Groom	Pre	4.4 ± 1.3	1.8 ± 1.2	1.2 ± 0.6	3.1 ± 1.0	0.9 ± 0.3	2.0 ±1.2
	$Threat_5$	4.8 ± 0.8	4.6 ± 0.7	**2.5 ± 0.7**	**2.6 ± 0.7**	**1.2 ± 0.7**	**1.2 ± 0.9**
	$Threat_{55}$	5.1 ± 1.5	3.6 ± 1.8	5.2 ± 1.8	2.3 ± 0.8	2.6 ± 1.2	0.6 ± 0.5
Diazepam (mg/kg); n=10		Vehicle		1.0	3.0	6.0	10.0
"high" USV	Pre	36.3 ± 15.7		27.5 ± 15.3	14.6 ± 7.5	**9.7 ± 7.0**	**5.6 ± 2.7**
	$Threat_5$	63.7 ± 23.8		69.1 ± 28.8	49.1 ± 26.4	34.9 ± 16.7	**31.3 ± 17.9**
	$Threat_{55}$	31.8 ± 11.8		18.2 ± 5.3	30.3 ± 18.8	22.6 ± 12.7	21.0 ± 16.0
Defensive upright	Pre	3.9 ± 1.3		1.5 ± 0.9	1.1 ± 0.9	**1.2 ± 0.9**	**0.2 ± 0.2**
	$Threat_5$	18.1 ± 3.3		20.8 ± 5.2	19.6 ± 4.2	16.4 ± 3.5	12.3 ± 2.9
	$Threat_{55}$	9.8 ± 2.4		7.0 ± 2.5	6.0 ± 2.4	4.9 ± 2.5	**2.0 ± 0.9**
Walk	Pre	5.4 ± 1.4		4.2 ± 1.7	4.3 ± 1.3	**2.0 ± 0.6**	**1.3 ± 0.9**
	$Threat_5$	5.2 ± 0.9		5.0 ± 1.4	**2.7 ± 0.5**	**1.8 ± 0.4**	**1.6 ± 0.5**
	$Threat_{55}$	1.9 ± 0.4		1.9 ± 0.5	1.6 ± 0.5	1.8 ± 0.5	**0.9 ± 0.4**
Crouch	Pre	36.5 ± 3.1		43.2 ± 5.4	42.6 ± 5.8	34.8 ± 6.3	37.2 ± 8.2
	$Threat_5$	28.7 ± 3.8		26.5 ± 4.9	**33.4 ± 4.5**	**39.6 ± 4.2**	**46.3 ± 2.4**
	$Threat_{55}$	37.8 ± 2.6		33.2 ± 6.3	37.0 ± 5.3	34.3 ± 5.3	46.1 ± 5.0
Lie	Pre	0.0 ± 0.0		0.0 ± 0.0	4.4 ± 4.4	7.1 ± 4.9	**18.3 ± 8.7**
	$Threat_5$	0.1 ± 0.1		0.5 ± 0.5	0.4 ± 0.3	1.0 ± 0.7	1.7 ± 1.0
	$Threat_{55}$	3.5 ± 1.0		7.2 ± 5.9	9.1 ± 4.3	16.0 ± 5.3	4.7 ± 3.4
Groom	Pre	5.4 ± 1.8		1.5 ± 0.8	1.4 ± 1.2	4.7 ± 4.6	0.0 ± 0.0
	$Threat_5$	3.4 ± 1.2		4.1 ± 1.3	**1.6 ± 0.6**	**0.5 ± 0.2**	**0.4 ± 0.2**
	$Threat_{55}$	4.8 ± 0.6		4.6 ± 1.7	4.3 ± 2.0	1.4 ± 0.7	4.2 ± 3.5
Ethanol (g/kg); n=12		Vehicle	0.1	0.3	1.0	1.7	3.0
"high" USV	Pre	10.8 ± 4.6	6.0 ± 4.3	3.4 ± 2.3	3.6 ± 2.6	**0.6 ± 0.3**	**0.5 ± 0.4**
	$Threat_5$	26.8 ± 9.7	17.1 ± 6.9	20.9 ± 10.0	27.7 ± 11.6	33.9 ± 17.1	44.0 ± 14.2
	$Threat_{55}$	15.3 ± 3.0	17.1 ± 6.4	**8.4 ± 3.0**	10.7 ± 3.1	**6.3 ± 2.2**	**4.8 ± 3.0**
Defensive upright	Pre	13.4 ± 3.4	12.5 ± 5.1	10.0 ± 4.7	6.5 ± 1.9	**3.3 ± 1.2**	**2.3 ± 1.0**
	$Threat_5$	17.0 ± 3.0	15.9 ± 4.3	14.1 ± 2.4	13.8 ± 2.7	12.1 ± 2.4	11.5 ± 1.8
	$Threat_{55}$	8.9 ± 2.5	6.8 ± 2.0	7.5 ± 2.0	10.3 ± 4.2	**5.0 ± 1.6**	**2.0 ± 0.7**
Walk	Pre	3.2 ± 0.8	2.7 ± 0.8	2.8 ± 1.0	2.3 ± 0.8	2.2 ± 0.6	3.3 ± 1.3
	$Threat_5$	5.0 ± 0.6	4.6 ± 0.7	4.6 ± 0.6	5.1 ± 0.8	5.4 ± 0.6	6.2 ± 0.8
	$Threat_{55}$	3.0 ± 0.4	3.6 ± 0.5	2.5 ± 0.4	3.6 ± 0.6	**2.3 ± 0.4**	2.7 ± 0.4
Crouch	Pre	37.4 ± 3.4	40.7 ± 4.8	43.9 ± 4.4	45.8 ± 2.3	47.0 ± 2.1	40.2 ± 5.1
	$Threat_5$	30.9 ± 2.5	34.4 ± 3.8	31.8 ± 1.8	33.6 ± 2.8	35.8 ± 2.2	36.9 ± 1.9
	$Threat_{55}$	40.5 ± 3.2	42.2 ± 2.4	39.4 ± 3.0	33.9 ± 3.6	31.2 ± 3.8	30.2 ± 3.6
Lie	Pre	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 1.2	2.4 ± 1.0	**12.6 ± 5.1**
	$Threat_5$	0.2 ± 0.1	0.3 ± 0.2	0.4 ± 0.2	2.0 ± 1.1	**3.8 ± 1.4**	**3.4 ± 1.2**
	$Threat_{55}$	4.7 ± 1.3	4.9 ± 2.2	5.7 ± 1.9	9.7 ± 3.2	**15.9 ± 4.5**	**20.6 ± 4.6**
Groom	Pre	3.1 ± 0.5	3.9 ± 1.3	3.5 ± 1.3	2.6 ± 1.1	1.1 ± 0.5	1.6 ± 0.8
	$Threat_5$	5.9 ± 1.1	5.1 ± 1.2	8.4 ± 2.2	5.3 ± 1.0	**3.7 ± 0.9**	**1.5 ± 0.8**
	$Threat_{55}$	1.6 ± 0.4	2.7 ± 1.2	4.5 ± 2.0	3.3 ± 1.2	2.7 ± 1.4	4.6 ± 2.4

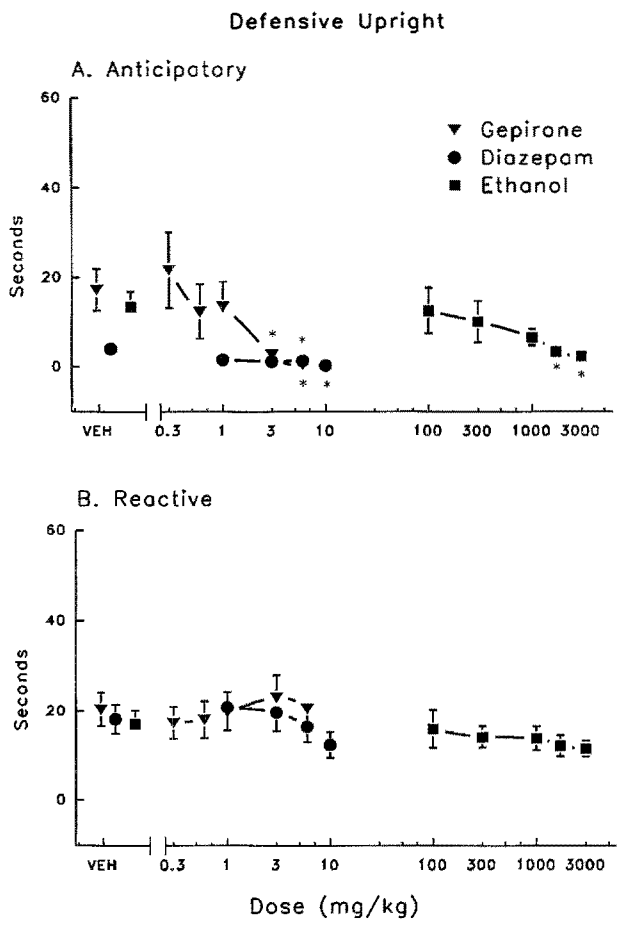

Fig. 3A, B Comparison of the effects of gepirone ($n = 11$), diazepam ($n = 10$) and ethanol ($n = 12$) on defensive behavior of defeat experienced intruder rats **A** prior to defeat and **B** at the beginning of the threat encounter. The durations of defensive upright are expressed per minute, means and SEM are given and *asterisks* mark mean values which are statistically different from the corresponding vehicle treatment ($P < 0.05$)

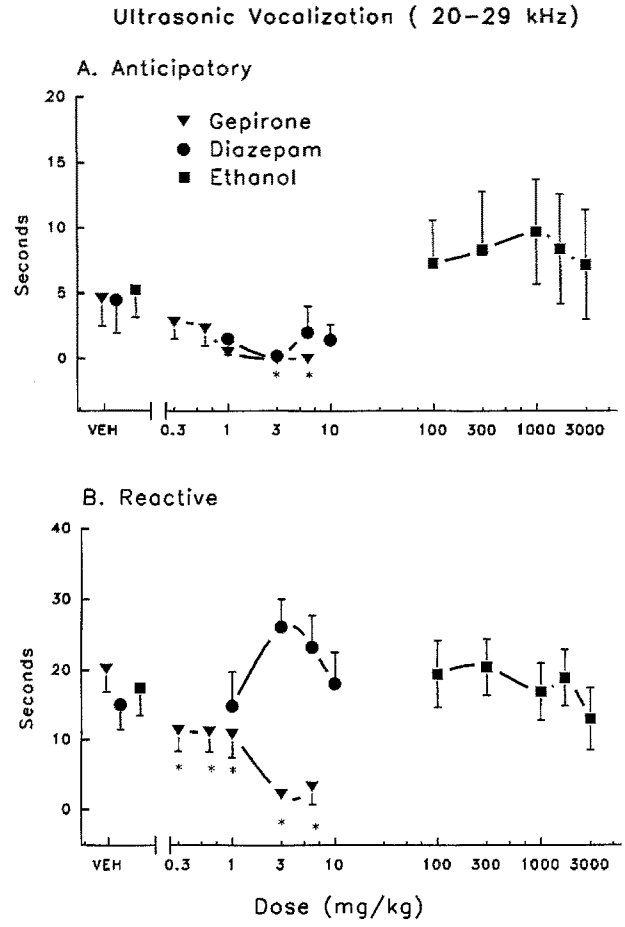

Fig. 4A, B Comparison of the effects of gepirone ($n = 11$), diazepam ($n = 10$) and ethanol ($n = 12$) on ultrasonic vocalizations emitted by defeat experienced intruder rats **A** prior to defeat and **B** at the beginning of the threat encounter. Note the different scaling of the y-axis. The durations of 20 to 31-kHz low-frequency USV are expressed per minute, means and SEM are given and *asterisks* mark mean values which are statistically different from the corresponding vehicle treatment ($P < 0.05$)

3.0 g/kg dose of ethanol consisted of the substantial increase in the time spent lying during all three periods of the confrontation [$F(5, 55) = 5.3$, $P < 0.01$; $F(5, 55) = 4.9$, $P < 0.01$; $F(5, 55) = 4.9$, $P < 0.01$; Table 2].

Changes in ultrasonic vocalizations (USV)

Gepirone dose-dependently decreased low-frequency USV throughout the social confrontation [prior to defeat: $F(5, 50) = 3.2$, $P < 0.02$; at the beginning: $F(5, 50) = 7.9$, $P < 0.001$; and at the end of the threat encounter: $F(5, 50) = 2.9$, $P = 0.02$]. The 3.0 and 6.0 mg/kg dose completely suppressed low-frequency USV in most of the animals. Diazepam and ethanol had no significant attenuating effects on these calls (Fig. 4). The rate of high-frequency USV prior to defeat was dose-dependently decreased by diazepam and ethanol but not by gepirone [see Table 2; $F(4, 36) = 3.6$, $P = 0.01$; $F(5, 55) = 2.6$, $P < 0.04$; $F(5, 50) = 1.9$, $P > 0.1$]. After defeat diazepam attenuated the calling rate of high-frequency USV at the beginning of the threat encounter [$F(4, 36) = 2.8$, $P < 0.05$], which was the period of the confrontation when the rate of USV emitted by the vehicle-treated intruders was highest (Table 2), whereas ethanol significantly decreased the rate of high-frequency USV at the end of the 1 hr threat encounter [$F(5, 55) = 3.5$, $P < 0.001$.

Discussion

The magnitude of changes in behavior and physiology of the intruder during consecutive phases of a confrontation with an aggressive resident increased, peaked and declined in an orderly manner. The anxiolytic-like effects of alcohol, diazepam and gepirone depended on the intensity of the demand on the intruder during the course of the confrontation. During phases of the confrontation with lower stress intensity such as

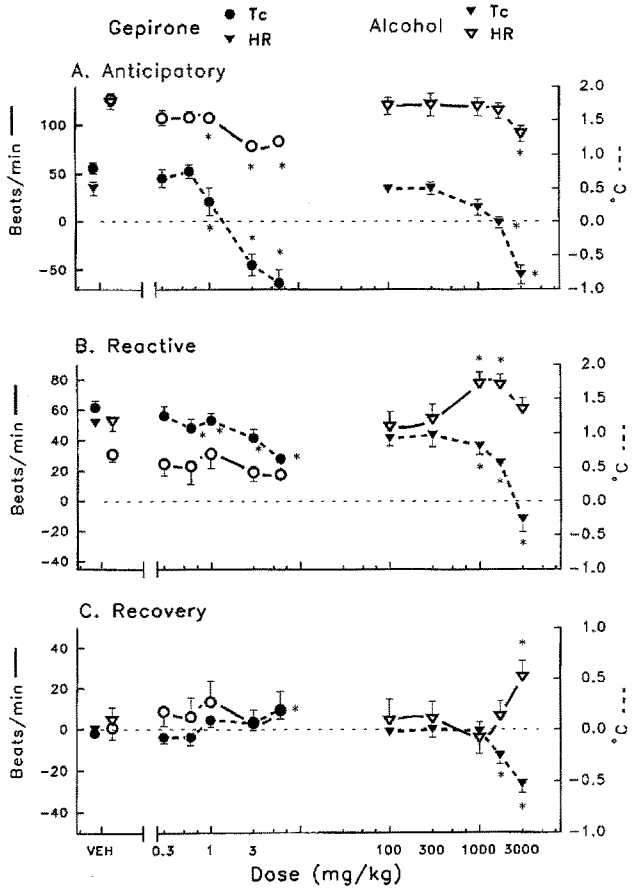

Fig. 5A–C Differential effects of gepirone ($n = 9$; *circles*) and ethanol ($n = 10$; *triangles*) on heart-rate (——) and core temperature (- - -) of defeat experienced intruder rats **A** prior to defeat, **B** during the threat encounter and **C** during recovery from social stress. For details see data analysis. Means and SEM of the deviations from baseline are given and *asterisks* mark mean values which are statistically different from the corresponding vehicle treatment ($P < 0.05$)

during the "anticipatory" phase, several physiological and behavioral "stress" reactions were attenuated by alcohol, diazepam or gepirone at non-sedative doses: hyperthermia, tachycardia, intensified defensive behavior and USV and suppressed locomotor and exploratory behavior. In contrast to this anxiolytic-like profile, none of these drugs was effective during the more intense reactive phase of the test, immediately following defeat. Even sedation and hypothermia, as induced by the higher doses of alcohol, did not change defensive responding and ultrasonic vocalization.

Social confrontations are significant events for the subordinate animal (Miczek et al. 1991a). The behavioral, autonomic and endocrine responses to social stress are larger and longer-lasting in the subordinate than in the aggressor (Barnett 1975; Schuurman 1980; Von Holst 1985; Raab et al. 1986; Fokkema et al. 1988). In intruder rats, for example, daily confrontations induced profound increases in defense, suppression in exploratory behavior, and a long-term decrease in amplitude of the circadian rhythms for HR and T_c (Tornatzky and Miczek 1993; Harper et al. 1995). Less frequent confrontations induced similar changes in the acute behavioral and physiological stress responses but did not induce the long-term debilitating changes in HR, blood pressure and T_C baselines or circadian rhythms (see Table 1, Tornatzky and Miczek 1994; Meehan et al. 1995). When the intruder is repeatedly defeated in confrontations with the resident, the hyperthermia, tachycardia, and defensive reactions during the "anticipatory" phase prior to defeat increased, while the exploratory and locomotor activity of the intruders decreased (Tornatzky and Miczek 1994).

The lack of anxiolytic-like effects of alcohol, diazepam and gepirone during the reactive phase could be due to the intensity and the proximity of the relevant social stressor. Even sedative doses of diazepam did not decrease low-frequency USV of defeat-experienced or -inexperienced rats in the most intense phase of the attack and threat encounter (see Fig. 4; Vivian and Miczek 1993). The β-blocker metoprolol, the α_2-adrenergic agonist clonidine, or alcohol did not decrease defensive behavior and low-frequency USV in the reactive phase of the confrontation (see Figs. 2 and 4; Tornatzky and Miczek 1994). Similarly, defensive behaviors of rats approaching or contacting predators remained largely unaffected by anxiolytics (Blanchard et al. 1990, 1993). However, in situations associated with the potential threat of the cat, benzodiazepines, $5HT_{1A}$-agonists and alcohol produced an anxiolytic-like profile in that they reduced suppression of feeding and drinking and decreased scanning of the "threat" area characterized by the "flat back" posture. The dependence of drug effects on stimulus control was previously established for neuroleptics in that chlorpromazine reduced avoidance during the signal for the impending electric shock, but left escape reactions to the shock intact (Cook and Weidley 1957).

The intensity of the social confrontation determines the effects of anxiolytics on ultrasonic vocalizations. They were attenuated by all three drugs in the "anticipatory" phase of the confrontation with diazepam and alcohol affecting high-frequency USV and gepirone affecting low-frequency USV (see Table 2 and Fig. 4). Only gepirone was effective in attenuating low-frequency USV prior to and following defeat which is consistent with similar effects described for $5\text{-}HT_{1A}$ agonists in other contexts (Winslow and Insel 1991; DeVry et al. 1993; Vivian and Miczek 1993). However, the potent decrease in low-frequency USV by gepirone during the reactive period of the confrontation in the absence of other behavioral indicators of anxiolytic-like activity should be interpreted as a selective effect on the production of vocalizations. Diazepam and even alcohol in a sedative dose-range are ineffective in ameliorating low-frequency USV in this period of intense social confrontation. The $5\text{-}HT_{1A}$-selective effects on facial

muscles (Berendsen et al. 1989), with the possible detrimental effects on the mechanisms involved in the production of these calls (Roberts 1975), has to be considered when USV are evaluated as the sole variable reflecting anxiolytic-like activity. The complete suppression of low-frequency USV by 3.0 and 6.0 mg/kg gepirone excludes the untreated resident as the source of these calls (see also Tornatzky and Miczek 1994).

The ready detection of potentially detrimental side-effects of anxiolytics is a major feature of the current method which focuses on a more comprehensive evaluation of the autonomic and behavioral profile. The lack of sedative side-effects of gepirone is one of the advantages described for 5-HT_{1A} agonists when compared to benzodiazepines and alcohol (see Table 2; Taylor DP et al. 1985; Hjorth and Carlson 1988; Pecknold 1994). Nevertheless, like diazepam and alcohol, gepirone decreased the intruder's hyperthermia during the course of the confrontation confirming the effects of these drugs in lowering core temperature (Peris and Cunningham 1985; Taylor SC et al. 1985; Goodwin et al. 1986; Murphy et al. 1991). Gepirone decreased the T_C during the "anticipatory" phase and to a lesser extent attenuated hyperthermia during the reactive period of the confrontation (see Fig. 5). Alcohol, on the other hand, had pronounced hypothermic effect throughout the confrontation (see Fig. 1). The potentiation of tachycardia during the threat period and the recovery period by the higher doses of alcohol may be interpreted as a compensatory cardiovascular mechanism which counteracts the homeostatic challenge caused by the drug induced hypothermia in this demanding period of the confrontation (see Fig. 5). Neither gepirone nor diazepam showed these long-term effects on autonomic measures. By contrast, a high clonidine dose compromises the organisms homeostasis, due to its pronounced sedative, hypothermic and bradycardic effects, and causes anxiogenic-like responses (e.g. increased low-frequency USV) during the reactive phase of the experiment (Tornatzky and Miczek 1994).

Pharmacologically, the closely similar anxiolytic-like effects during the "anticipatory" phase of the confrontation by agents with diverse mechanisms of action prompt the speculation for a common pathway (Miczek et al. 1995). Whether 5-HT_{1A} receptors and the benzodiazepine-$GABA_A$ receptor complex are in fact the critical mechanisms for the currently observed effects of gepirone, diazepam and alcohol has to await appropriate antagonism studies.

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References

Adams DB, Baccelli G, Mancia G, Zanchetti A (1968) Cardiovascular changes during preparation for fighting behaviour in the cat. Nature 220:1239–1240

Aston-Jones S, Aston-Jones G, Koob GF (1984) Cocaine antagonize anxiolytic effects of ethanol. Psychopharmacology 84:28–31

Barnett SA (1975) The rat. A study in behavior. University of Chicago Press, Chicago

Barrett JE, Brady LS, Witkin JM (1985) Behavioral studies with anxiolytic drugs. I. Interactions of the benzodiazepine antagonist Ro 15–1788 with chlordiazepoxide, pentobarbital and ethanol. J Pharmacol Exp Ther 233:554–559

Becker HC, Hale RL (1991) RO15-4513 antagonizes the anxiolytic effects of ethanol in a nonshock conflict task at doses devoid of anxiogenic activity. Pharmacol Biochem and Behav 39:803–807

Berendsen HHG, Jenck F, Broekkamp LE (1989) Selective activation of 5HT1A receptors induces lower lip retraction in the rat. Pharmacol Biochem Behav 33:821–827

Blackburn JR, Phillips AG, Fibinger HC (1987) Dopamine and preparatory behavior: I. Effects of pimozide. Behav Neurosci 101:352–360

Blanchard DC, Blanchard RJ, Tom P, Rodgers RJ (1990) Diazepam changes risk assessment in an anxiety/defense test battery. Psychopharmacology 101:511–518

Blanchard DC, Veniegas R, Elloran I, Blanchard RJ (1993) Alcohol and anxiety - effects on offensive and defensive aggression. J Stud Alcohol Suppl. 11:9–19

Blanchard RJ, Blanchard DC, Agullana R, Weiss SM (1991) Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. Physiol Behav 50:967–972

Borsini F, Lecci A, Volterra G, Meli A (1989) A model to measure anticipatory anxiety in mice? Psychopharmacology 98:207–211

Browne LJ, Shaw KJ (1991) New anxiolytics. Annu Rep in Med Chem 26 26:1–10

Chopin P, Briley M (1987) Animal models of anxiety: the effect of compounds that modify 5-HT neurotransmission. Trends Pharmacol Sci 8:383–388

Cook L, Weidley E (1957) Behavioral effects of some psychopharmacological agents. Ann NY Acad Sci 66:740–752

DeVry J, Benz U, Schreiber R, Traber J (1993) Shock-induced ultrasonic vocalization in young adult rats: A model for testing putative anti-anxiety drugs. Eur J Pharmacol 249:331–339

Eisermann K (1992) Long-term heart rate responses to social stress in wild european rabbits: predominant effect of rank position. Physiol Behav 52:33–36

Estes WK, Skinner BF (1941) Some quantitative properties of anxiety. J Exp Psychol 29:390–400

File SE (1985) Animal models for predicting clinical efficacy of anxiolytic drugs: social behaviour. Neuropsychobiology 13:55–62

Fokkema DS, Smit K, Van der Gugten J, Koolhaas JM (1988) A coherent pattern among social behavior, blood pressure, corticosterone and catecholamine measures in individual male rats. Physiol Behav 42:485–489

Goodwin GM, De Souza RJ, Green AR (1986) The effects of a 5HT receptor ligand isapirone (TVX Q 7821) on 5HT synthesis and the behavioral effects of 5HT agonists in mice and rats. Psychopharmacology 89:382–387

Grant EC, MacKintosh JH (1963) A comparison of the social postures of some common laboratory rodents. Behaviour 21:246–259

Green S (1991) Benzodiazepines, putative anxiolytics and animal models of anxiety. Trends Neurosci 14:101–104

Hajos M, Engberg G (1986) Emotional hyperthermia in spontaneously hypertensive rats. Psychopharmacology 90:170–172

Haney M, Miczek KA (1993) Ultrasounds during agonistic interactions between female rats (*Rattus norvegicus*). J Comp Psychol 107:373–379

Harper DG, Tornatzky W, Miczek AM (1995) Stress induced disorganization of circadian and ultradian rhythms: comparisons of effects of surgery and social stress. Physiol Behav: (in press)

Henry JP, Liu YY, Nadra WE, Qian CG, Mormede P, Lemaire V, Ely D, Hendley ED (1993) Psychosocial stress can induce chronic hypertension in normotensive strains of rats. Hypertension 21:714–723

Hjorth S, Carlson A (1988) Buspirone effects on central monoaminergic transmission: Possible relevance to animal experimental and clinical findings. Eur J Pharmacol 83:299–303

Hommer DW, Skolnick P, Paul SM (1987) The benzodiazepine/GABA receptor complex and anxiety. In: Meltzer HY (ed) Psychopharmacology: the third generation of progress. Raven Press, New York, pp 977–983

Insel TR, Ninan PT, Aloi J, Jimerson DC, Skolnick P, Paul SM (1984) A benzodiazepine receptor-mediated model of anxiety. Arch Gen Psychiatry 41:741–750

Kalin NH, Shelton SE (1989) Defensive behavior in infant Rhesus monkeys: environmental cues and neurochemical regulation. Science 243:1718–1721

Kaltwasser MT (1990) Acoustic signaling in the black rat. J Comp Psychol 104:227–232

Koob GF, Thatcher-Britton K (1985) The role of GABA/benzodiazepine complex in the anxiolytic actions of ethanol. Alcohol Clin Exp Res 9:204

Koob GF, Braestrup C, Britton KT (1986) The effects of FG 7142 and RO 15–1788 on the release of punished responding produced by chlordiazepoxide and ethanol in the rat. Psychopharmacology 90:173–178

Lal H, Emmett-Oglesby MW (1983) Behavioral analogues of anxiety–animal models. Neuropharmacology 22:1423–1441

Meehan WP, Tornatzky W, Miczek KA (1995) Blood pressure via telemetry during social confrontations in rats: Effects of clonidine. Physiol Behav (in press)

Miczek KA (1979) A new test for aggression in rats without aversive stimulation: differential effects of *d*-amphetamine and cocaine. Psychopharmacology 60:253–259

Miczek KA (1982) Ethological analysis of drug action on aggression, defense and defeat. In: Spiegelstein MY, Levy A (eds) Behavioral models and the analysis of drug action. Elsevier, Amsterdam, pp 225–239

Miczek KA, Vivian JA (1993) Automatic quantification of withdrawal from 5-day diazepam in rats: ultrasonic distress vocalizations and hyperreflexia to acoustic startle stimuli. Psychopharmacology 110:379–382

Miczek KA, Thompson ML, Tornatzky W (1991a) Subordinate animals: behavioral and physiological adaptations and opioid tolerance. In: Brown MR, Koob GF, Rivier C (eds) Stress: neurobiology and neuroendocrinology. Marcel Dekker, New York, pp 323–357

Miczek KA, Tornatzky W, Vivian JA (1991b) Ethology and neuropharmacology: rodent ultrasounds. In: Olivier B, Mos J, Slangen JL (eds) animal models in psychopharmacology. Birkhauser, Basel, pp 409–427

Miczek KA, Weerts EM, Tornatzky W, DeBold JF, Vatne TM (1992) Alcohol and "bursts" of aggressive behavior: ethological analysis of individual differences in rats. Psychopharmacology 107: 551–563

Miczek KA, Weerts E, Vivian JA, Barros H (1995) Aggression, anxiety and vocalizations in animals: $GABA_A$ and 5-HT anxiolytics. Psychopharmacology: (in press)

Mistlberger RE (1992) Anticipatory activity rhythms under daily schedules of water access in the rat. J Biol Rhythms 7:149–160

Murphy DL, Lesch KP, Aulakh CS, Pigott TA (1991) Serotonin-selective arylpiperazines with neuroendocrine, behavioral, temperature, and cardiovascular effects in humans 3. Pharmacol Rev 43:527–552

Pecknold JC (1994) Serotonin $5\text{-}HT_{1A}$ agonists: a comparative review. CNS Drugs 2:234–251

Peris J, Cunningham CL (1985) Dissociation of tolerance to the hypothermic and tachycardic effects of ethanol. Pharmacol Biochem Behav 22:973–978

Pfaus JG, Phillips AG (1991) Role of dopamine in anticipatory and consummatory aspects of sexual behavior in the male rat. Behav Neurosci 105:727–743

Poncelet M, Dangoumau L, Soubrie P, Simon P (1987) Effects of neuroleptic drugs, clonidine and lithium on the expression of conditioned behavioral excitation in rats. Psychopharmacology 92:393–397

Raab A, Dantzer R, Michaud B, Mormede P, Taghzouti K, Simon H, LeMoal M (1986) Behavioural, physiological and immunological consequences of social status and aggression in chronically coexisting resident- intruder dyads of male rats. Physiol Behav 36:223–228

Roberts LH (1975) The rodent ultrasound production mechanism. Ultrasonics 13:83–88

Sanger DJ, Perrault G, Morel E, Joly D, Zivkovic B (1991) Animal models of anxiety and the development of novel anxiolytic drugs. Prog Neuropsychopharmacol Biol Psychiatry 15:205–212

Schuurman T (1980) Hormonal correlates of agonistic behavior in adult male rats. In: McConnel PS, Boer GJ, Romijn HJ, Van de Poll NE (eds), Progress in brain research, vol. 53: adaptive capabilities of the nervous system (vol. 53). Elsevier Biomedical Press, Amsterdam, pp 415–420

Sumova A, Jakoubek B (1989) Analgesia and impact induced by anticipation stress: involvement of the endogenous opioid peptide system. Brain Res 503:273–280

Taylor DP, Moon SL (1991) Buspirone and related compounds as alternative anxiolytics. Neuropeptides 19:15–19

Taylor DP, Eison MS, Riblet LA, Van der Maelen CP (1985) Pharmacological and clinical effects of buspirone. Pharmacol Biochem Behavior 23:687–694

Taylor SC, Little HJ, Nutt DJ, Sellars N (1985) A benzodiazepine agonist and contragonist have hypothermic effects in rodents. Neuropharmacology 24:69–73

Ticku MK, Mhatre M, Mehta AK (1992) Modulation of GABAergic transmission by ethanol. In: Biggio G, Concas A, Costa E (eds) GABAergic synaptic transmission. Raven Press, New York, pp 255–268

Tornatzky W, Miczek KA (1993) Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. Physiol Behavior 53:983–993

Tornatzky W, Miczek KA (1994) Behavioral and autonomic responses to intermittent social stress: differential effects of clonidine and metoprolol. Psychopharmacology 116:346–356

Van der Poel AM, Miczek KA (1991) Long ultrasonic calls in male rats following mating, defeat and aversive stimulation: Frequency modulation and bout structure. Behaviour 119:127–142

Vivian JA, Miczek KA (1993) Diazepam and gepirone selectivly attenuate either 20–32 or 32–64 kHz ultrasonic vocalizations during aggressive encounters. Psychopharmacology 112:66–73

Von Holst D (1985) Coping behaviour and stress physiology in male tree shrews. In: Hoelldobler B, Lindauer M (eds) Experimental behavioral ecology and sociobiology (vol. 31). Gustav Fischer, Stuttgart, pp 461–470

Weerts EM, Tornatzky W, Miczek KA (1993) "Anxiolytic" and "anxiogenic" benzodiazepines and beta-carbolines: effects on aggressive and social behavior in rats and squirrel monkeys. Psychopharmacology 110:451–459

White NR, Cagiano R, Moises AU, Barfield RJ (1990) Changes in mating vocalizations over the ejaculatory series in rats. J Comp Psychol 104:255–262

Wilkinson L (1987) SYSTAT: The system for statistics. Systat Evanston

Wilkinson LO, Dourish CT (1991) Serotonin and animal behavior. Serotonin Receptor Subtypes 15:147–210

Winslow JT, Insel TR (1991) The infant rat separation paradigm - A novel test for novel anxiolytics. Trends Pharmacol Sci 12:402–404