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Diazepam and gepirone selectively attenuate either 20–32 or 32–64 kHz ultrasonic vocalizations during aggressive encounters

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Abstract. Ultrasonic vocalizations (USV) in rats may communicate "affective" states, as they occur only in highly significant behavioral contexts such as during sex, aggression, exposure to painful or startling events. This proposal was evaluated in an experiment with adult male Long-Evans rats during agonistic encounters; specifically, the effects of diazepam, flumazenil and gepirone were studied on different types of USV emitted by intruder rats exposed to resident attacks and to "threat of attacks" (i.e., intruder protected within the home cage of the resident by a wire mesh cage). USV were readily emitted during agonistic encounters and consisted primarily of two distributions of pure tone whistles: 0.3- to 3-s, 20- to 32-kHz ("low") signals and 0.02- to 0.3-s, 32- to 64-kHz ("high") signals. A considerable repertoire of frequency modulated signals was observed and proved to be sensitive to the anxiolytic treatments. Diazepam (1–6 mg/kg) dose-dependently decreased high frequency USV during the threat of attack and decreased the mean pitch of the most predominant vocalizations but did not affect low frequency USV or the audible squeals (AS) in response to bites. Gepirone (0.3–6 mg/kg) dose-dependently decreased low frequency USV and did not affect high frequency USV or AS. Responses to thermal pain stimuli remained unaltered by all drugs, while walking duration was decreased and crouch postures were increased after diazepam but not after gepirone administration. Gepirone in the present dose range had minimal effects on submissive, exploratory and locomotor behaviors. The pattern of results is consistent with the proposal that low frequency USV reflect a heightened affective state which is ameliorated with $5HT_{1A}$ but not benzodiazepine anxiolytics, and suggests that the suppression of high frequency USV in reaction to attacks or threats coincides with the sedative or muscle relaxant properties of these compounds.

Key words: Ultrasonic vocalization – Diazepam – Gepirone – Anxiolytics – Aggression – Affect

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Ultrasonic vocalizations (USV) emitted by various rodent species are modulated by drugs acting at benzodiazepine and serotonin receptors among other neurochemical systems. These vocalizations may communicate the "affective state" of the sender. In addition to pharmacological evidence, support for a link between USV and affect is marshalled as USV are only emitted during stressful situations such as exposure to painful stimuli (Van der Poel et al. 1989), predation (Shepherd et al. 1992) and drug withdrawal (Vivian and Miczek 1991; Miczek and Vivian 1993). In pups, USV serve as stimuli for maternal retrieval and it has been proposed that USV may communicate "distress" (Nyby and Whitney 1978; Smith 1979); these distress vocalizations show promise as a screen for anxiolytic compounds (Gardner 1985; Winslow and Insel 1991b). The investigation of the subordinate animal – specifically the audible (AV) and ultrasonic vocalizations (USV) produced by the subordinate animal – during agonistic interactions may provide a novel and useful model for the study of "affective" expressions in an ethologically valid context that may be relevant to anxiety or more intense reactions in humans.

When threatened and attacked, subordinate rats rely on defensive and protective behaviors such as upright, supine and crouch postures and are relatively inactive (Rodgers and Hendrie 1983; Miczek et al. 1991). Similar to open-field experiments in which increased freezing or immobility and decreased exploration are interpreted to reflect heightened "emotionality," subordinate animals quickly refrain from maladaptive activities such as increased locomotor activity and exploration in the presence of an attacking conspecific (Miczek et al. 1990, 1991). Interestingly, USV responses, which have so far not been extensively examined in subordinate animals during agonistic encounters, coincide with decreased activity. Increases in USV are paralleled by marked cardiovascular and thermoregulatory deviations from homeostasis. Neither USV nor defensive and submissive behavior is affected by diazepam; clonidine, an $alpha_1$-adrenergic receptor agonist with anti-stress effects, actually increases these types of vocalizations (Tornatzky and

Miczek 1990). Ipsapirone, a newer anxiolytic with affinity for $5HT_{1A}$ receptors, also decreases exploration while increasing immobility behavior similar to morphine, diazepam and clonidine (Korte et al. 1990). These provocative findings prompted a more detailed investigation of the pharmacological modulation of USV as a potential indicator of "heightened emotionality" in rats.

In pups, the benzodiazepine receptor agonists diazepam and chlordiazepoxide consistently decrease USV emitted as a result of a cold environment, rough handling or administration of pentylenetetrazole at "anxiogenic" doses (Gardner 1985; Insel et al. 1986; Nastiti et al. 1991). Conversely, benzodiazepine inverse agonists such as DMCM, FG 7142 and CGS 8216 can increase or even elicit ultrasound production without affecting body temperature or producing sedation (Gardner and Budhram 1987; Nastiti et al. 1991). Further support for a benzodiazepine influence on pup USV is demonstrated as both benzodiazepine receptor agonist and inverse agonist effects are reversed with the antagonists flumazenil and ZK 93426 (Nastiti et al. 1991).

In adults, diazepam and alprazolam, but not desipramine or haloperidol, decreased USV duration in response to footshock (Tonoue et al. 1986; Cuomo et al. 1988). Recently, USV have been observed in response to acoustic and tactile startle stimuli (Miczek and Vivian 1993; Kaltwasser 1990a, 1991). Diazepam abolished USV rate and withdrawal from diazepam further potentiates USV production in response to acoustic startle stimuli (Miczek and Vivian 1993). The findings that acute diazepam reduces and diazepam withdrawal increases USV in response to startle stimuli and footshock are consistent with the view that USV are indicative of "anxiety".

Similarly, serotonin modulation of the production of USV has been documented in pups exposed to cold ambient temperatures. $5HT_{1A}$ agonists such as 8-OH-DPAT, buspirone, ipsapirone and gepirone, the $5HT_2$ agonist DOI, as well as reuptake inhibitors including clomipramine, fluvoxamine and zimelidine all reduce the rate of pup USV (Hard and Engel 1988; Mos et al. 1989; Winslow and Insel 1990). Further support for a serotonin modulation of pup USV includes increases in vocalizations with potential antagonists at the $5HT_{1B}$ receptor including TFMPP and CGS12066B (Winslow and Insel 1991a).

In adults, Mos et al. (1991) report decreases in post-ejaculatory USV with the selective $5HT_{1A}$ agonist 8-OH-DPAT; however, this effect coincided with decreases in the number of mounts and intromissions before ejaculation. Shock-, startle-, and aggression-elicited USV are also markedly attenuated by ipsapirone and gepirone (Vivian and Miczek, in preparation; Kaltwasser 1991; Tornatzky and Miczek 1991; Schreiber and De Vry 1993); these decreases have been interpreted as an indication of the anti-anxiety effects of $5HT_{1A}$ ligands. Interestingly, the $5HT_3$ antagonist ondansetron has been ineffective in reducing startle-elicited USV in both naive and diazepam-withdrawn subjects (Vivian and Miczek, in preparation).

The current experiments focus on two major sets of objectives: (1) to determine which type of USV are most sensitive to prototypic compounds which act at benzodiazepine and $5HT_{1A}$ receptors; are the drug effects related to escape, immobility and analgesia? (2) to extend the investigation of USV through a more detailed analysis of the frequency characteristics which may differentiate the variety of sounds and their differential sensitivity to anxiolytic challenges.

Materials and methods

Subjects. One hundred and four experimentally naive male hooded Long-Evans rats (Charles River Labs, Wilmington, MA) weighing 300–400 g served as subjects. They were housed individually in clear polycarbonate cages ($48 \times 27 \times 20$ cm^3) with stainless steel lids. Additional male Long-Evans hooded rats weighing 550–650 g were used as stimulus males ("residents") for agonistic encounters and were housed with female Long-Evans rats (250–400 g) in $45.7 \times 45.7 \times 71.1$ cm^3 stainless steel cages (Vivian and Miczek 1993). All animals were housed in a vivarium with controlled light (12/12 h light/dark cycle), temperature (21 ± 1°C) and humidity (30–40%); they had free access to Purina rodent chow pellets and water.

Apparatus. USV were recorded with a condensor microphone (Bruel & Kjaer Type 4135), preamplifier (Bruel & Kjaer Type 2633), filter (Krohn-Hite Model 3550R; nominal settings: 20–70 kHz, bandpass) and amplifier (Bruel & Kjaer Type 2610) onto tapes (Maxell UD 35–90N) using an eight-channel instrumentation recorder (Hewlett-Packard Model 3968A).

USV were analyzed using two systems. (1) Trained listeners depressed keys in response to signals made audible via quarter speed taped playback through an amplifier into headphones (Radio Shack Model 33–2002) and concurrently displayed on a spectrum analyzer (Tektronix Model 5L4N). This playback provided rate, duration, onset and offset times of USV, and these data were summarized with customized software (Princeton Economics, Princeton, MA). (2) Sonographs of the first 20 low and 20 high frequency USV for each diazepam subject were digitized and displayed with hardware/software developed for the Apple MacIntosh computer (MacSpeech Lab II; GW Instruments, Cambridge, MA); this analysis determined beginning, end and modal frequency (kHz) of individual calls. Sonographic analysis also verified that none of the calls overlapped in time, i.e., they were not emitted by two different animals.

Behavioral measurements were conducted under infrared light and recorded with an infrared-sensitive camera (Canon Model Ci20R) in conjunction with a VHS video cassette recorder. Trained observers depressed keys in response to the behavioral events displayed on a video monitor and included social, locomotor and exploratory behaviors: walk, rear, inactivity, ano-genital and nasal contact, defensive behaviors: escape, upright, and submissive behaviors: crouch, supine, following the operational definitions of Miczek (1979). The frequency and duration of each behavioral event were summarized with customized software (Princeton Economics, Princeton, MA).

Experimental design and procedure. Baseline latencies for the tail flick reflex in response to a thermal pain stimulus were determined (d'Amour and Smith 1941), followed by drug administration. A second pain response latency was determined after 25 min in their home cage. Subsequently, subjects were placed into the home cage ($45.7 \times 45.7 \times 71.1$ cm^3) of a stimulus male for an agonistic encounter until definitive signs of submission were observed ("Attack Encounter"). Submissive signs included upright, crouch and supine postures with audible and ultrasonic vocalizations. When a supine posture was displayed for 5 consecutive seconds, the intruder was protected from the resident by a protective cage ($17.8 \times 17.8 \times 33.0$ cm^3) for the following 25 min ("Threat of Attack Encounter"). This protective cage prevented tactile contact between the two rats but permitted auditory, visual and olfactory contact.

Audio and video records were obtained during the exposure to attack and threat of attack at 5 and 25 min (2-min samples).

Drugs. Diazepam (1, 3, 6, 10 mg/kg; Hoffman LaRoche) or flumazenil (3, 10, 30 mg/kg; Hoffman LaRoche) was administered intraperitoneally 25 min prior to agonistic encounters. Gepirone (0.3, 1, 3, 6 mg/kg; Bristol-Myers) was administered intraperitoneally 20 min prior to agonistic encounters. Diazepam and flumazenil were prepared in a solution containing 85% distilled water, 14% propylene glycol, 1% Tween 80. Gepirone was dissolved in 0.9% physiological saline. All drugs were administered in a volume of 1 ml/kg body weight.

Data analysis. Behavioral data during the threat of attack at 5 and 25 min were collapsed after they were analyzed for within-subjects biases; no significant effects were noted. Behavioral response measurements were analyzed with separate one-factor (Drug) between-subjects ANOVAs. When significant effects were present, post-hoc Dunnett analyses were performed. In the analysis of frequency modulated USV and frequency step calls (see below), chi-square analyses were performed. When significant effects were present, post-hoc *z*-tests for proportions (Kachigan 1986) were performed. Differences were considered as significant when $P<0.05$, using the two-tailed criterion.

Results

Attack encounter

Characteristics of aggressive behavior by the stimulus animal. Diazepam, gepirone and flumazenil administered to the intruder were largely without effect on the following characteristics of the resident rat's attack behavior: attack latency, bites, aggressive postures or attack duration. On average, intruder subjects were attacked within ca. 30 s upon introduction into the resident's cage and received 14 bites in 44 s. Diazepam (6 mg/kg) decreased and gepirone tended to decrease the fight duration [$F(3,28)=3.01$, $P<0.05$; $F(4,35)=2.18$, $P<0.09$] and flumazenil (3.10 mg/kg) decreased the duration of aggressive postures [$F(3,27)=6.48$, $P<0.01$].

Audible and ultrasonic vocalizations. Audible squeals (AS) and USV were emitted by all rats, particularly after the first attack bite. AS were 0.1- to 3-s broad-band signals while pure tone USV were emitted primarily in two frequency ranges: 20- to 32-kHz (low USV) and 32- to 64-kHz (high USV) pulses. The greatest proportion of these high frequency USV were emitted between 38 and 52 kHz.

Diazepam and flumazenil were without effect on the rate and duration of AS, low and high frequency USV. Even at behaviorally sedating doses of diazepam, the subjects continued to vocalize. Similarly, gepirone was without effect on USV, but greatly enhanced AS (258%) at 6 mg/kg [$F(4,35)=3.75$, $P<0.01$].

Defensive and locomotor behavior. Diazepam decreased the duration of upright postures at 3.0 mg/kg [$F(3,28)=3.16$, $P<0.05$], but similar to flumazenil, produced negligible effects on the duration of crouch and supine postures, walking or rearing behavior. Gepirone produced no effect on the duration of any defensive or locomotor behaviors.

Threat of attack

Stimulus resident behavior. Diazepam, gepirone and flumazenil administered to the intruder produced no significant alterations in the duration of the resident's walking, being on top of the cage and attacking the cage behavior.

Audible and ultrasonic vocalizations. USV during the threat of attack were emitted as pure tone pulses within two frequency distributions: lower 20- to 32-kHz and higher 32- to 64-kHz signals. There were no AS during threat of attack encounters.

Diazepam and flumazenil did not alter the rate or duration of low frequency USV. Diazepam dose-dependently decreased the rate of high frequency USV [$F(3,28)=6.69$, $P<0.01$; Fig. 1], while flumazenil also decreased the rate of high frequency USV at 3 and 30 mg/kg [$F(3,27)=4.52$, $P<0.01$; Table 1]. In contrast, gepirone dose-dependently decreased the rate and duration of low frequency USV [$F(4, 35)=6.77$, $P<0.01$; $F(4, 35)=10.72$, $P<0.01$] without affecting high frequency USV. Pulse durations of individual calls were not affected by diazepam or gepirone.

Analysis of the effects of diazepam on pitch and frequency modulation of 1138 low and 422 high frequency

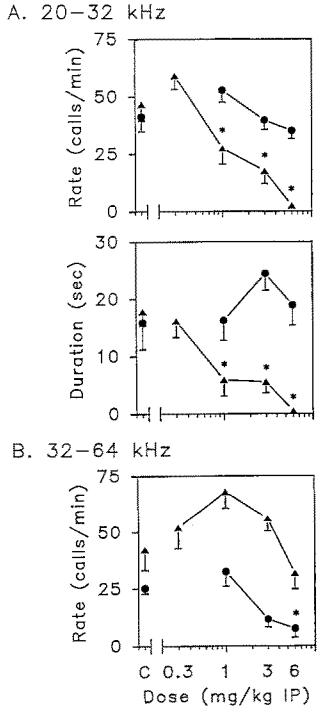

Fig. 1 A, B. The effects of diazepam and gepirone on **A** mean rate (calls/min) and duration (per min) of 20- to 32-kHz USV, and **B** rate (calls/min) of 32- to 64-kHz USV during threat of attack. *Asterisks* denote significant differences ($P<0.05$) from control; error bars indicate SEM. ●, Diazepam; ▲, gepirone

Table 1. Effects of flumazenil on motor, defensive and vocal behavior during the threat of attack

	Flumazenil (mg/kg)			
	0	3	10	30
Crouch				
Rate	0.72±0.24	3.13±0.50	1.50±0.41	1.79±0.41
Duration	12.25±3.63	42.34±2.91*	15.00±4.54	27.93±5.55
Walk				
Rate	5.94±1.14	2.13±0.77	3.50±0,88	2.14±1.00
Duration	10.75±1.20	3.24±1.33	8.54±2.21	4.03±2.09
Low frequency USV				
Rate	41.25±6.44	33.56±4.57	46.75±9.32	35.43±7.27
Duration	15.86±4.63	28.86±2.99	21.50±4.62	27.50±4.72
High frequency USV				
Rate	25.13±2.25	6.13±2.85*	25.88±7.73	4.21±1.63*

Rate and duration of behavioral events are expressed per minute
* Indicates $P<0.05$ compared to control

USV was also performed, as well as diazepam's effect on ten distinctive call types (for details, see Vivian and Miczek 1993). For low frequency USV, three calls were observed to be the most prevalent: linear decelerating (LD: 27.2% of all calls), monotonic (M: 19.2%) and hyperbolic (H: 25.1%; Fig. 2). For LD calls, there was a modest 8% increase in the proportion of occurrence with the highest doses of diazepam. Diazepam also produced a biphasic effect on the beginning, end and modal pitch: 1 mg/kg increased beginning, end and modal pitch while 3 and 6 mg/kg decreased them [beginning: $F(3,305)=18.42$, $P<0.01$, end: $F(3,305)=14.16$, $P<0.01$ and modal: $F(3,305)=20.66$, $P<0.01$]. The proportion of M and H calls was largely unaffected by diazepam administration; however, similar to LD calls, 1 mg/kg diazepam increased beginning and modal pitch while 3 and 6 mg/kg decreased these characteristics [beginning M: $F(3,215)=16.22$, $P<0.01$ and modal M: $F(3,215)=17.15$, $P<0.01$, beginning H: $F(3,282)=25.89$, $P<0.01$, modal H: $F(3,282)=10.52$, $P<0.01$; Table 2].

For high frequency USV, LD (11.4% of all calls), M (18.5%) and linear accelerating (LA: 21.3%) calls were the most prevalent. Diazepam increased the proportion of LD calls at 3 mg/kg [$\chi^2(3)=15.28$, $P<0.01$] while concurrently decreasing the beginning, end and modal pitch at 3 and 6 mg/kg [beginning: $F(3,44)=18.06$, $P<0.01$, end: $F(3,44)=14.59$, $P<0.01$ and modal: $F(3,44)=16.75$, $P<0.01$]. Alternatively, the occurrence and pitch characteristics of M calls were unaffected by diazepam administration. Although the occurrence of LA calls was unaffected by diazepam, there was a dose-dependent decrease in beginning, end and modal pitch which reached significance at 3 mg/kg [beginning: $F(3,86)=6.14$, $P<0.01$, end: $F(3,86)=9.56$, $P<0.01$ and modal: $F(3,86)=5.53$, $P<0.01$]. The effects of diazepam on high frequency USV are detailed in Table 3.

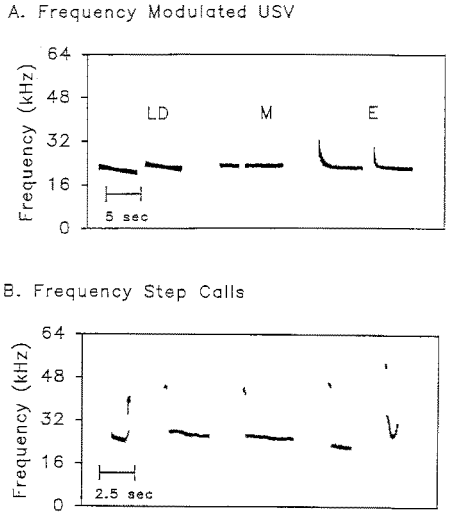

Fig. 2. A Spectrograms of frequency modulated 20- to 32-kHz USV produced during threat of attack. *LD*, linear decelerating; *M*, monotonic; *H*, hyperbolic; **B** Spectrograms of frequency step calls

In the analysis of frequency step calls (Fig. 2; Kaltwasser 1991b), diazepam produced biphasic effects on the occurrence of high frequency components emitted before the lower frequency component [$\chi^2(3)=63.09$, $P<0.01$]. Diazepam (1 mg/kg) produced an 18% increase in the occurrence of this type of calls while 3 and 6 mg/kg decreased their occurrence by 10 and 13%, respectively. High frequency components emitted after the low frequency components were dose-dependently decreased by diazepam; this decrease was significant at 3 and 6 mg/kg [$\chi^2(3)=25.21$, $P<0.01$].

Table 2. Effects of diazepam on pitch of frequency modulated ultrasounds during threat of attack. Low frequency ultrasounds

	Diazepam (mg/kg)			
	0	1.0	3.0	6.0
Linear decelerating				
Beginning	30607±1191	32727±985	25242±558*	26042±676*
End	26024±725	28257±887*	23079±468*	23253±562*
Modal	24812±361	26620±339*	23033±302*	23318±375*
Monotonic				
Beginning	25797±718	31322±1112*	24303±515	24468±9188
End	28633±1147	27727±823	23796±477*	23737±9234*
Modal	24869±462	26304±438*	23285±324*	22662±3714*
Hyperbolic				
Beginning	31797±1039	35011±1125*	28194±553*	25422±352*
End	23778±703	26518±800*	23700±388	22366±649
Modal	22530±281	24038±306*	23465±339*	21589±332*

Frequency values are expressed in Hz

* Indicates $P<0.05$ compared to control

Table 3. Effects of diazepam on pitch of frequency modulated ultrasound during threat of attack. High frequency ultrasounds

	Diazepam (mg/kg)			
	0	1.0	3.0	6.0
Linear decelerating				
Beginning	51058±1644	47013±1228	38320±905*	40095±2743*
End	48495±1920	44687±1034	35917±1078*	40718±2863*
Modal	49487±1900	45823±1179	36594±1003*	38729±2564*
Monotonic				
Beginning	43707±1187	44893±1203	40914±915	43769±2404
End	43959±1279	45065±1208	41419±992	44277±2547
Modal	43724±1234	44213±1134	41110±939	44167±2519
Logarithmic				
Beginning	38681±1332	41748±653	40576±544	41252±927
End	47329±1565	46409±796	43950±654	47214±1131
Modal	44068±1430	46271+854	43842±762	45952±1156
Linear accelerating				
Beginning	43858±867	43185±770	39065±822*	39337±1019
End	48219±679	48215±822	42624±846*	45298±1758
Modal	46207±715	45488±904	41546±772*	43298±1536

Frequency values are expressed in Hz

* Indicates $P<0.05$ compared to control

Defensive and locomotor behavior. Diazepam dose-dependently increased crouch duration [$F(3,28)=6.59$, $P<0.01$], with concomitant dose-dependent decreases in the duration of walking [$F(3,28)=8.49$, $P<0.01$; Fig. 3]. These alterations in defensive and locomotor behavior were not accompanied by changes in nasal orientation. The effects of flumazenil were variable, leaving orienting, defensive and locomotor behavior unaltered. There was an elevation of crouch behavior at 3 mg/kg but not at 10 or 30 mg/kg [$F(3,27)=6.15$, $P<0.01$; Table 1]. Gepirone was without effect on any component of locomotor or defensive behavior.

Tail flick reflex to thermal pain. Diazepam, flumazenil and gepirone all failed to alter the latency to remove the tail from a heat stimulus.

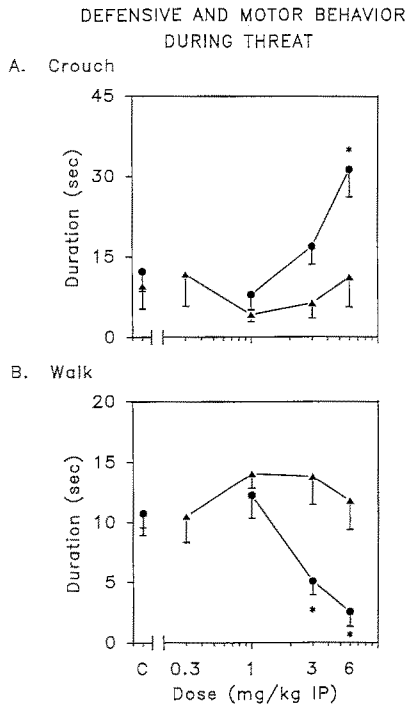

Fig. 3A, B. The effects of diazepam and gepirone on **A** the duration (per min) of crouch posture, and **B** the duration (per min) of walking behavior. *Asterisks* denote significant differences ($P < 0.05$) from control; error bars denote SEM. ● Diazepam; ▲ gepirone

Discussion

Gepirone was quite potent and selective in attenuating the production of low frequency USV during the threat of attack while measures of pain reflexes, locomotor and defensive behaviors were not altered. Nonetheless, the proposal that the production of USV during agonistic encounters communicates an "affective" state was not entirely supported as diazepam was ineffective in reducing low frequency USV in rats that were attacked and threatened, and did not reliably reduce high frequency USV during exposure to attack. The mu opiate receptor agonist morphine decreased both low and high frequency USV (Vivian and Miczek 1993). These results suggest that low frequency USV communicate the affective state of the intruder while the attenuation of high frequency USV is consistent with the muscle relaxant or sedative properties of these compounds.

Clinical anxiety disorders are composed of several types of anxiety, such as generalized or anticipatory anxiety disorders which are ameliorated with diazepam, and panic attacks which are less effectively treated with this drug (Baldessarini 1985). It is possible that the attack or threat of attack exposures engender a more intense reaction (i.e., "panic-" or "depressed-" like states which are not benzodiazepine-receptor sensitive; although see Liebowitz et al. 1986) and is indicated by the inability of diazepam to reduce low frequency USV.

Although benzodiazepines influence both pup and adult USV (Vivian and Miczek, in preparation; Gardner 1985; Insel et al. 1986; Kaltwasser 1991), many of these experimental situations do not engender high frequency USV. Less sedative or muscle relaxant anxiolytics such as bretazenil (Martin et al. 1988) may reveal if high frequency USV provide another measure of sedation. Further research should also include compounds useful in the treatment of panic disorder and depression (i.e., tricyclic antidepressants, fluoxetine or monoamine oxidase inhibitors; see Liebowitz et al. 1985; Klein et al. 1987) to address whether low frequency USV during agonistic encounters are pertinent to "panic-" or "depression-" like phenomena in adult rats. If low frequency USV are related to these affective states, these compounds should decrease USV production. Interestingly, antidepressant activity with buspirone and gepirone has been reported (Taylor 1990), while the treatment of panic has been unsuccessful (Charney et al. 1990).

Another important determinant for pharmacological modulation of USV is the intensity of the eliciting stimulus. In the current experiment, 100% of the intruder subjects vocalized when confronted with an attacking conspecific, yet diazepam was ineffective in attenuating low frequency USV. However, diazepam in non-sedative doses *is* quite effective in reducing low frequency USV in intruders with defeat experience when they are exposed to the environment of the attack without the presence of the aggressor (Tornatzky and Miczek 1990). In experiments involving electric shock, approximately 60–80% of the subjects emit USV. Interestingly, benzodiazepine receptor agonists are effective in reducing USV as a result of foot shock, but are ineffective in tail shock paradigms (Tonoue et al. 1986; Cuomo et al. 1988; Van der Poel et al. 1989). Finally, USV are produced by 50–60% of the subjects exposed to acoustic startle stimuli and appear to be exquisitely sensitive to benzodiazepine and $5HT_{1A}$ receptor agonists: doses as low as 1 mg/kg for diazepam and 0.6 mg/kg for gepirone produced reliable decreases (greater than 66% reduction) in USV production without alterations in startle nor analgesia measurements (Vivian and Miczek, in preparation). Therefore, it appears that the intensity of the eliciting stimuli (i.e., defeat > shock > startle) determines the sensitivity of USV to pharmacological challenges; these vocalizations continue to reflect the "affective state" of the animal, but are not necessarily limited to the expression of anxiety-like states.

In the pitch domain, diazepam had very consistent biphasic effects on low frequency calls: 1 mg/kg elevated the pitch of LD, M and H calls while 3 and 6 mg/kg decreased their pitch. These changes are consistent with, but not solely due to, the expression of frequency step calls (1 mg/kg diazepam increased, 3 and 6 mg/kg decreased their production). It is unclear what the changes in pitch convey; interestingly, increases in pitch, pitch variability and intensity of vocal characteristic *in humans* are associated with heightened emotionality (Scherer and Kappas 1988), therefore diazepam's suppression of USV pitch is consistent with the hypothesis that lower pitched USV are produced during a lessened emotional state. The suggestion that frequency step calls are associated with catalepsy (Kaltwasser 1990b) is not supported by the present results. Diazepam, at 3 and 6 mg/kg de-

creased the occurrence of both types of frequency step calls while concurrently increasing the duration of the crouch posture. Finally, intruders are the sole source of USV because: (1) low and high frequency USV are emitted when defeat-experienced intruders are exposed to the environment of the attack *without the presence of the aggressor* (Tornatzky and Miczek 1990), and (2) no USV were detected at any time in which the resident was alone in the experimental cage (e.g., immediately prior to or after the attack or threat of attack exposure).

Diazepam had little influence on the display of defensive and locomotor behaviors during attack and threat of attack up to the highest and sedating dose. Except for a decrease in the duration of walking and an increase in crouch behaviors, diazepam was without effect on orienting, upright, rearing, and escape activity. These findings are concordant with the observations that nondefensive measures of locomotor behavior in rats (i.e., rearing, grooming) were inconsistently altered by diazepam (Rodgers and Randall 1987; Blanchard et al. 1989, 1990a). Depending on the intensity of the "anxiety"-producing stimulus, increases and decreases were observed on defensive measures including crouch postures (Blanchard et al. 1990b). Tornatzky and Miczek (1990, 1991) observed similar diazepam outcomes: higher doses produced sedation and were without effect on defensive behavior in drug- and attack-experienced subjects.

Defensive, locomotor and pain reflex measures were relatively unaffected by gepirone administration; in contrast to the sedative effects of diazepam, these effects are not a feature of gepirone and related $5HT_{1A}$ anxiolytics (Taylor 1990). Similarly, flumazenil did not alter analgesia, and had inconsistent effects on locomotor and defensive behaviors; doses lower than 1.25 mg/kg may decrease defensive behaviors in mice, while doses of 2.5–20 mg/kg were ineffective (Rodgers and Waters 1984, 1985). Finally, the finding that benzodiazepine receptor agonists and antagonists and $5HT_{1A}$ agonists did not influence the tail flick reflex was expected (Morgan et al. 1987a,b; Rodgers and Shepherd 1989).

The current results reveal that the emerging characterization of USV and their link to affective functioning requires more than the examination of rate and duration measures. Low frequency USV during the threat of attack are selectively attenuated by gepirone and morphine and may reveal that *low frequency USV* are more relevant than high frequency USV in interpretations of affect as engendered by aggressive encounters (Vivian and Miczek 1993). In contrast, suppression of high frequency USV in reaction to intense threats and attacks are achieved only at muscle relaxant or sedative dose of both diazepam (current experiment) and morphine (Vivian and Miczek 1993). Further support for the hypothesis that low frequency USV communicate the affective state of the intruder emerges from the analysis of the pitch characteristics of individual types of calls. Similar to morphine, diazepam decreased the pitch of the most predominant types of calls, indicating that a reduction in "emotionality" is discernible with a more refined analysis of USV.

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