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REVIEW

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Aggression, anxiety and vocalizations in animals: $GABA_A$ and 5-HT anxiolytics

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Abstract A continuing challenge for preclinical research on anxiolytic drugs is to capture the affective dimension that characterizes anxiety and aggression, either in their adaptive forms or when they become of clinical concern. Experimental protocols for the preclinical study of anxiolytic drugs typically involve the *suppression* of conditioned or unconditioned social and exploratory behavior (e.g., punished drinking or social interactions) and demonstrate the reversal of this behavioral suppression by drugs acting on the benzodiazepine-$GABA_A$ complex. Less frequently, aversive events engender *increases* in conditioned or unconditioned behavior that are reversed by anxiolytic drugs (e.g., fear-potentiated startle). More recently, putative anxiolytics which target 5-HT receptor subtypes produced effects in these traditional protocols that often are not systematic and robust. We propose ethological studies of vocal expressions in rodents and primates during social confrontations, separation from social companions, or exposure to aversive environmental events as promising sources of information on the affective features of behavior. This approach focusses on vocal and other display behavior with clear functional validity and homology. Drugs with anxiolytic effects that act on the benzodiazepine-$GABA_A$ receptor complex and on 5-HT_{1A} receptors systematically and potently alter specific vocalizations in rodents and primates in a pharmacologically reversible manner; the specificity of these effects on vocalizations is evident due to the effectiveness of low doses that do not compromise other physiological and behavioral processes. Antagonists at the benzodiazepine receptor reverse the effects of full agonists on vocalizations, particularly when these occur in threatening, startling and distressing contexts. With the development of antagonists at 5-HT receptor subtypes, it can be anticipated that similar receptor-specificity can be established for the effects of 5-HT anxiolytics.

Key words Anxiolytics · Benzodiazepines · 5-HT agonists · Benzodiazepine receptors · 5-HIAA · Aggression · Punishment · Startle · Vocalizations · Exploratory behavior · Social behavior · Dominance · Maternal behavior · Pain · Defense · Stress · Opiates · Ultrasounds

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Introduction

The neurobiological and psychopharmacological study of aggression and anxiety is faced with the initial challenge of how to relate clinical and social concerns with pathological excesses to our understanding of these behaviors' ontogenetic and phylogenetic adaptive origin. Clinicians and public health officials focus on an excess or, alternatively, a lack of anxious and aggressive behavior, while ethological analyses delineate the adaptive nature of these behavior patterns. The most global definitions of aggression and anxiety attempt to encompass adaptive as well as pathological forms. Alternatively, psychiatric and ethological definitions are offered that emphasize either the pathological extremes of aggression and anxiety or, alternatively, refer to their evolutionary roots and importance in the survival of the individual and the species. One important dimension of aggressive and anxious behavior patterns that has eluded adequate quantitative assessment in clinical, as well as preclinical research, is the affective or emotional nature of these activities. Here we propose that certain types of vocalizations may represent

affective expressions that are accessible to quantitative behavioral and neuropharmacological preclinical investigations.

Clinical and preclinical traditions

The nosological situation is considerably more satisfactory for "anxiety" than for "aggression" in clinical populations (Eichelman and Hartwig 1993). The Diagnostic and Statistical Manual of Mental Disorder IV (DSM IV) of the American Psychiatric Association (1994) clearly distinguishes several types of anxiety disorders, such as panic disorder, phobic disorder (e.g., agoraphobia, social phobia, and simple phobia), obsessive-compulsive disorder, post-traumatic stress disorder, generalized anxiety disorder (GAD), and organic anxiety syndrome. By contrast, the preclinical protocols have not achieved this degree of differentiation. In addition, anxiety can appear concurrently with other pathological states such as alcoholism, depression disorders, schizophrenia and organic brain disorders. Here we focus on benzodiazepine and 5-HT_{1A} anxiolytics that are most effective in GAD (e.g., Lader and Petursson 1981; Greenblatt et al. 1983; De Vry 1995).

By contrast, aggression remains unrecognized as a diagnostic category in the DSM IV (American Psychiatric Association 1994), but aggressive behavior appears as a symptom in several disorders that include: mental retardation, attention deficit disorder, organic mental syndromes, schizophrenia, delusional disorder, brief reactive psychosis, mood disorders, anxiety disorder, dissociative disorders, impulse control disorder, adjustment disorder and personality disorders (Eichelman 1987). Mysteriously, while ultimately every behavior is a function of brain activity, the recent public debate in the US reveals considerable reluctance in the social science community to relate aggressive behavior to any neural function. On the other hand, preclinical research of aggressive behavior has been guided mainly by the objectives and methods of biomedical, social and ethological scientific traditions.

From a biomedical perspective, *aggression* under inappropriate conditions is considered a *disease* caused by disordered neural activity that needs to be treated (Mark and Ervin 1970). Aggressive outbursts are part of the symptomatology of a range of psychiatric and neurological diseases (vide supra). In spite of many clinical case reports, it still remains poorly understood how, for example, seizure disorders are related to aggressive outbursts, and whether or not the neurobiological mechanisms for ictal and interictal events are related to those for explosive aggressive behavior (Eichelman 1983). In fact, the limbic dyscontrol syndrome has been suggested to encompass both aggressive and seizure events (e.g., Monroe 1978). There is some success with the anticonvulsants phenobarbital or carbamazepine in the reduction of aggressive behavior (Tardiff 1983; Munizz et al. 1993). The historic Phineas Gage episode points to traumatic and toxic insults to the brain as sources for aggressive episodes (Siegal and Mirsky 1994).

The approach in the social sciences highlights environmental determinants of aggressive behavior and refers to *aggression* as *antisocial* behavior (e.g., Berkowitz 1993). Here, the causative factors are assigned to the distal living conditions, such as isolated, impoverished or crowded housing, or to proximal triggers, such as noxious and aversive stimuli. For more than 50 years, the omission of scheduled reinforcement or "frustrative non-reward" has been a prime example for aggression as antisocial activity (Dollard et al. 1939).

On the other hand, the ethological objectives in the study of aggressive behavior seek to understand the evolutionary origin and functional significance of these behavior patterns (Lorenz 1966; Marler 1976). The *adaptive significance* of aggressive behavior in reproductive contexts is apparent in dominance, territorial and maternal aggression when potential rivals or intruders are repulsed and the brood is defended (Huntingford and Turner 1987; Archer 1988).

Preclinical research strategies for psychopharmacological and neurobiological investigations of aggressive and anxious behavior patterns are primarily guided by pharmacological validation, with homologies across species remaining elusive. While aggressive and anxious behaviors are characterized by affective or emotional features, this dimension is rarely part of preclinical psychopharmacological research. In fact, most research during the past three decades suggests that anxiolytics with sites of action at the $GABA_A$-benzodiazepine receptor complex and, more recently, the 5-HT_{1A} receptor profoundly and systematically modulate animal behaviors that represent adaptations to aversive environmental events.

Major preclinical research methods for predicting anxiolytic effects

The many preclinical tests or "models" for the study of anxiolytic drugs can be grouped according to the type of experimental manipulations, the nature and direction of the behavioral change, and the resulting effects as predicting anxiolytic effects. Based on the effects of prototypic benzodiazepine drugs, the preclinical tests predict anxiolytic activity either by (1) restoring behavior that is suppressed by aversive contingencies, (2) attenuating behavior that is increased by aversive manipulations, or (3) reversing a pharmacologically induced "anxiogenic" state (e.g., File 1985; Handley 1991). Table 1 compares the effects of chlordiazepoxide and diazepam as prototypic benzodiazepines with the preclinical work on the potentially anxiolytic effects of buspirone as the first clinically

Table 1 Preclinical methods predicting anxiolytic effects – acute prototypic effects

Method	Benzodiazepine anxiolytics				5-HT agonists			
	Drug	Dose (mg/kg)	Effect	Reference	Drug	Dose (mg/kg)	Effect	Reference
Restoration of reversal of behaviors suppressed by aversive contingency								
C Punished Operant Response	CDP	3–10 (P)	↑	Barrett (1976)	BUS	0.1–40 (R,P)	0/↑	Barrett and Witkin (1991)
		3–30 (PG)	↑	Barrett et al. (1986)	BUS	0.001–0.3 (PG)	↑	Barrett et al. (1986)
U Plus-maze Open-arm entry	DZP	1–25	↑	Klint (1991)	BUS	1	↓	Klint (1991)
	CDP	1–7.5	↑		BUS	0.8	↑	File and Andrews (1991)
					BUS	0.1–0.4	0	File and Andrews (1991)
Suppression of behaviors increased by aversive stimulus								
C Fear-potentiated startle	DZP	0.3–2.5	↓	Davis (1979)	BUS	0.6–10	↓	Davis (1986)
U Defensive burying	DZP	0.5–2	↓	Treit et al. (1981)	BUS	0.05–1	↓	Treit and Fundytus (1988)
Drug-induced anxiogenic states								
C PTZ-discrimination	DZP	5–10	↓	Lal and Shearman (1982)	BUS	0.56 (IM)(P)	↑	Ator et al. (1989)
					BUS	0.56–1	0	Ator (1991)
U Anxiety-like responses	DZP	1–2	↓	Ninan et al. (1982)				

CDP chlordiazepoxide, *DZP* diazepam, *BUS* Buspirone. All doses refer to intraperitoneal administration, unless stated: (IM) intramuscular route; (IV) intravenous route. *C* conditioned behavior, *U* unconditioned behavior, *R* rats, *M* mice, *P* primates, *PG* pigeons

introduced serotonergic agent. Major reviews have summarized the effects of drugs that act at least partially as 5-HT_{1A} receptor agonists in procedures that were developed for the benzodiazepine anxiolytics, i.e. that either (1) suppress behavior as a result of aversive contingencies or (2) engender behavior due to aversive stimulation (Chopin and Briley 1987; Barrett and Witkin 1991; Barrett and Vanover 1993; Handley and McBlane 1993; Handley et al. 1993).

Restoration or reversal of behavior that is *suppressed* by aversive consequences

The classic preclinical findings were obtained with procedures that highlighted how benzodiazepines reversed the behaviorally suppressive effects of punishment such as those introduced by Geller and Seifter (1960), and by Vogel et al. (1971). For example, Sepinwall and Cook demonstrated systematic, dose-dependent benzodiazepine effects on punishment-suppressed responding in squirrel monkeys that clearly differ from those on concurrently assessed non-punished responding (Sepinwall and Cook 1984). Of course, these procedures require considerable conditioning of the animal's behavior in order to obtain stable baselines of punished and unpunished responding. Yet, with a few well-trained animals systematic, dose-dependent, repeatable punishment-attenuating effects are seen with all benzodiazepine anxiolytics. The punishment-attenuating effects of full or partial benzodiazepine agonists are attenuated by antagonists such as flumazenil (Liljequist and Engel 1984).

So far, the most persuasive preclinical evidence on systematic, reliable, dose-dependent attenuation of punishment-suppressed behavior by drugs with affinity for 5-HT_{1A} receptors emerged from studies with pigeons, whereas similar studies in rats and primates remain only partially successful (Barett 1992; Sanger 1992). Buspirone, an anxiolytic with some affinity for 5-HT_{1A} receptors, increases specifically punishment-suppressed responding without significantly affecting non-punished food-reinforced key-pecking in pigeons (Barrett and Witkin 1991). An agent with a more selective affinity for the 5-HT_{1A} receptor subtype, flesinoxan, proved to be also highly effective in increasing food-reinforced responding that was suppressed by punishment (Barrett et al. 1989). Notably, when given chronically, buspirone effectively attenuates punishment-suppressed behavior even in rats (Schefke et al. 1989). A critical next step will be the reversal of these agonist effects by newly developed so-called silent antagonists in order to implicate the 5-HT_{1A} receptor more adequately in these behavioral effects.

In a research method that does not require conditioning, the focus is on exploratory behavior. In Montgomery's plus maze, rats readily enter into walled arms, but rarely into open arms of the apparatus (Montgomery 1955). This procedure does not require any training, but necessitates relatively inexperienced subjects for each test. Usually, behavioral specificity is assessed separately by measuring benzodiazepines'

effects on motor activity. Pellow et al. (1985) demonstrated how benzodiazepines increase the time that rats spend in the open arms of a plus-maze which is interpreted to reflect anxiolytic effects of these drugs. Flumazenil antagonizes the benzodiazepine effect on open arm entries (Wada and Fukuda 1991; Rex et al. 1993).

Similarly, buspirone and gepirone increase the time spent in the usually avoided open arm of a plus-maze, (Söderpalm et al. 1989). Similar effects have been reported for ondansetron, a drug acting at 5-HT_3 receptors (Costall et al. 1988, 1989) but this effect appears less robust (File and Johnston 1989).

Attenuation of behavior that is *increased* by aversive manipulations

When exposed to certain aversive conditions animals display characteristic responses, some of them require conditioning such as the "fear"-potentiated startle, others simply measure how much effort is exerted to remove the aversive, noxious stimulus such as in a rat's "defensive burying."

In the "fear"-potentiated startle procedure, increases in the startle reflex are measured when the presentation of a light that has previously been paired repeatedly with electric shock, immediately precedes a loud acoustic stimulus. Benzodiazepines are among the drugs that attenuate this increase in a dose-dependent fashion without altering the non-potentiated startle reflex (Davis 1979). The antagonist flumazenil blocks the diazepam effect on potentiated startle suggesting the benzodiazepine receptor as the relevant site of action for this effect (Berg and Davis 1984).

Partial and full 5-HT_{1A} receptor agonists have also effectively attenuated the "fear"-potentiated startle. For example, Buspirone and gepirone decreased in a dose-dependent, systematic manner the potentiated startle reflex (Kehne et al. 1988). So far, however, attempts to block buspirone effects with a range of serotonergic antagonists have been unsuccessful. Moreover, the prototypic 5-HT_{1A} agonist 8-OH-DPAT, over a range of several doses, did not alter fear-potentiated startle, suggesting non-serotonergic mediation (Davis et al. 1988).

Benzodiazepines suppress burying behavior of an electrified probe in rats that have been shocked previously by the probe (Treit et al. 1981). This effect appears to be mediated via the benzodiazepine receptor, since flumazenil blocked the suppressive effects of chlordiazepoxide (Treit 1987). Similar to the benzodiazepines, buspirone decreased defensive burying of the shock probe (Treit and Fundytus 1988), although these effects depend on specific testing parameters (Craft et al. 1988).

Anxiolytics with different mechanisms of action appear to selectively attenuate conditioned and unconditioned behavior that is engendered or amplified by exposure to aversive environmental events; these effects occur at non-sedative doses and without apparent development of tolerance (Sepinwall and Cook 1984; although see, for example, Vellucci and File 1979).

Attenuation of drug-induced "anxiogenic" state

A so-called anxiogenic state can be induced by pharmacological agents of several classes. Some procedures require elaborate conditioning such as the drug discrimination procedure, others simply engender a behavioral, physiological and neurochemical profile of "anxiety" responses as a result of drug administration.

Lal and associates trained rats to discriminate pentylenetetrazol from saline in a two-lever choice task (Lal et al. 1988). The injection of either PTZ or saline served as the discriminative stimulus or "cue" defining which response on one of two levers was reinforced, i.e. "drug-appropriate". After the rats responded more than 90% of the time on the drug-appropriate lever, they were pretreated with benzodiazepine anxiolytics and demonstrated a systematic, dose-dependent attenuation of the PTZ discriminative stimulus. Moreover, animals that are withdrawn from morphine, cocaine, alcohol or benzodiazepines generalize to the previously established PTZ discriminative stimulus, presumably on the basis of their withdrawal distress (Emmett-Oglesby et al. 1983, 1984; Lal et al. 1988; Wood et al. 1989). Recently, this pharmacological method to induce an anxiety-like state has attained considerable face-validity. For example, rats that are exposed to a predator or to an attacking or threatening conspecific generalize this state to the previously conditioned PTZ discriminative stimulus (Vellucci et al. 1988; Gauvin and Holloway 1991; Vivian et al. 1994b). Most saline-injected rats, when exposed to an attacking or threatening opponent, respond on the pentylenetetrazol-appropriate operandum. Midazolam pretreatment prevents the substitution of the social stress for the PTZ stimulus (Vellucci et al. 1988; Vivian et al. 1994b).

In chaired macaque monkeys, the IV injection of β-CCE induced behavioral changes that included agitated responses and distress vocalizations (Ninan et al. 1982). These responses correlated with endocrine measures of stress and were attenuated by benzodiazepines and other anxiolytics (Crawley et al. 1985). While some behavioral features of these β-CCE-treated monkeys are reminiscent of macaques that are threatened (Redmond and Huang 1979), the prerequisite of adaptation to chair-restraint for a stress-free baseline renders this experimental protocol in terms of face-validity.

To summarize, benzodiazepines consistently and effectively attenuate the suppressive effects of punishment on conditioned and unconditioned behavior in various animal species including humans. Similarly, these drugs reduce so-called fear- or stress-induced

conditioned and unconditioned responses. Drug-induced "anxiogenic" states such as the pentylenetetrazol "cue" or the β-CCE behavioral and physiological responses are attenuated by benzodiazepines. These anxiolytics achieve their behavioral effects via benzodiazepine receptors which is demonstrated by the reversal of the behavioral and physiological responses by receptor antagonist administration. These observations in animals point to the early recognition of the anxiolytic effects of benzodiazepines through the use of an impressive range of procedures. While generally validated with benzodiazepines through the use of an impressive range of procedures. While generally validated with benzodiazepine agonists and antagonists, the available experimental protocols differ considerably in terms of face validity, economy of training time and animal use. The implicit assumption for attenuating and reversing behavioral changes by benzodiazepine anxiolytics is that these behavioral effects are due to changes in affective or emotional processes.

Partial and full $5HT_{1A}$ agonists are characterized by systematic and potent effectiveness only in some of the "classic" punishment and fear-conditioning procedures (e.g., Przegalinski et al. 1992), but as will be discussed in detail below, more convincingly in those experimental procedures that engender so-called fear or distress responses such as in rat pups separated from their mother and nest, in adult rats that are threatened by conspecifics, or that are in withdrawal "distress"; only few preclinical studies have given these drugs repeatedly or chronically which is necessary for buspirone and ipsapirone to become effective in ameliorating GAD in humans (e.g., Pecknold 1994).

Most current experimental preparations have been effective predictors for treatment of GAD, but have been less successful for identifying drugs for the other types of anxiety. GAD, obsessive-compulsive disorders and panic disorders often benefit from different pharmacological treatments and appear to have different neurobiological mechanisms requiring separate preclinical behavioral procedures.

Major experimental methods for studying the effects of anxiolytics on aggression

The research strategies for delineating the role of 5-HT in aggressive behavior in animals and in clinical populations differ substantially from those for characterizing the benzodiazepines' effects on these behavior patterns. While many studies on 5-HT have relied upon correlating an index of serotonergic activity in brain tissue, blood or CSF with an "aggressive" trait, benzodiazepine research has developed a profile of drug effects on dominance, territorial and maternal aggression as well as defensive reactions to attacks by rivals and predators. Illustrative evidence from these latter procedures is summarized in Table 2.

Table 2 Experimental methods for studying the effects of anxiolytics on aggression. *CDP* chlordiazepoxide, *DZP* diazepam, *ALP* alprazolam, *BUS* Busirone, *GEP* Gepirone, *ELT* Eltoprazine, *8-OH-DPAT* 8-hydroxy-dipropylaminotetralin. All doses refer to intraperitoneal administration, unless stated: (PO) oral route; (SC) subcutaneous route. IM intramuscular route

Method	Benzodiazepine anxiolytics				5-HT agonists			
	Drug	Dose (mg/kg)	Effect	Reference	Drug	Dose (mg/kg)	Effect	Reference
Dominance (rats)								
	CDP	2.5–5	↑	Miczek, (1974)	BUS	2–8	↓	Mos et al. (1993)
		20(IM)	↓	Miczek, (1974)	8-OH-DPAT	0.1–0.2 (SC)	↓	
					ELT	1.25–5-	↓	
Territorial (mice)								
a) Male-female housing	CDP	5	↑	Miczek and O'Donnell, (1980)	BUS	1–10	0	Gao (1993)
		20	↓		GEP	3–30	↓	Miczek, unpublished
b) Single-housed	CDP	5–20	↓	Rodgers and Waters, (1985)	BUS	1–10	↓	Olivier et al. (1989)
					8-OH-DPAT	1–10		
Maternal aggression (rats)								
	ALP	1.2	↑	Mos and Olivier (1989)	BUS	0.3–3	0	Mos et al. (1992)
		5	↓					
	CDZ	5–20 (PO)	↑		8-OH-DPAT	0.1–0.2	↓	
	DZP	0.3–1	↑	Olivier et al. (1991)				
		3–17	↓		ELT	2–8	↓	
Defense								
Intruder rats	DZP	3–10	↓/0	Vivian and Miczek (1993a) Tornatzky and Miczek (1995)	GEP	3–6	↓/0	Vivian and Miczek, (1993a) Tornatzky and Miczek (1995)

Benzodiazepines

Benzodiazepine effects have been studied on all major types of aggressive behavior in animals (Table 2; e.g., Miczek and Krsiak 1979; Rodgers and Waters 1985; Miczek 1987; Mos et al. 1987; Sulcova and Krsiak 1989; Krsiak and Sulcova 1990). Diazepam and other sedative-hypnotics such as alcohol share the feature of increasing aggressive behavior by resident rats confronting an intruder, when given at lower doses (Miczek 1974; Miczek and Barry 1977; Olivier et al. 1984; Olivier et al. 1991; Miczek et al. 1992); however, at higher doses, these drugs decrease aggressive behavior, primarily due to their sedative and muscle-relaxant effects. The aggression-heightening effects of alcohol can be antagonized by benzodiazepine receptor antagonists in resident rats and in dominant squirrel monkeys, but the aggression-decreasing effects remain unaltered by these antagonists (Weerts et al. 1993b). This is illustrated in alcohol-treated dominant squirrel monkeys that display aggressively and emit aggressive vocalizations toward lower ranking group members or rivals (Weerts and Miczek, unpublished).

In singly or pair-housed mice that confront intruders into their territory, chlordiazepoxide and midazolam increase aggressive behavior at low doses and decrease it at higher doses (Miczek and O'Donnell 1980; Rodgers and Waters 1985). Similarly, in certain mice, particularly those with elevated levels of testosterone, low alcohol doses increase aggressive behavior, whereas higher doses decrease it (DeBold and Miczek 1985; Miczek et al., submitted). Antagonism and receptor binding studies point to the $GABA_A$-benzodiazepine receptor complex as the most likely site for the alcohol aggression-heightening effects (e.g., Weerts et al. 1992; Grant 1994; Miczek et al. 1994a).

Similarly, the biphasic nature of the benzodiazepine effect on aggression is seen in maternal female rats and mice (Olivier et al. 1985; Yoshimura 1987). The increase in aggressive behavior is evident as more frequent pursuits, threats and attacks after administration of low doses of benzodiazepines. Curiously, flumazenil failed to antagonize the increased aggressive behavior of lactating female rats after chlordiazepoxide treatment (Mos et al. 1987).

In contrast to the aggression-enhancing effects of benzodiazepines, these drugs dose-dependently attenuate flight responses in selectively bred timid mice (Krsiak 1975) and defensive responses in feral rats (Blanchard et al. 1989). In feral rats, intermediate doses of benzodiazepines specifically reduce defensive threat and attack without altering the remainder of the agonistic repertoire. The antagonist flumazenil attenuated the benzodiazepine inhibition of flight behavior in mice (Sulcova and Krsiak 1984).

To summarize, benzodiazepines exert biphasic effects on aggressive behavior by a resident male confronting a territorial intruder, by rivals in dominance contests or by a lactating female defending her litter, i.e. lower doses increased aggressive behavior and higher doses decreased these behaviors. Defensive responses to conspecifics and predators are routinely decreased by benzodiazepines, although only at intermediate and higher doses that are sedative.

Benzodiazepines' and alcohol's enhancement of aggressive behavior have been attributed to a disinhibitory action similar to the release of inhibition of punished behavior. However, the similarity appears to be only superficial. First, in rats, the doses that are sufficient to increase aggressive behavior are almost tenfold lower than those necessary to increase punished behavior. Also, increases in aggressive behavior after benzodiazepine treatment are not universal. For example, recently we observed large increases in low-rate operant behavior after alprazolam administration, but concurrent decreases in aggressive behavior in the same mice (Miczek, unpublished observations). In order to maintain a disinhibition hypothesis as a unifying principle for benzodiazepine effects on suppressed behavior and on aggressive behavior, it is necessary to postulate multiple inhibitory mechanisms, each dependent on the $GABA_A$-benzodiazepine receptor complex.

5-HT drugs

No other neurotransmitter system has been more frequently linked to a broad range of aggressive behavior than serotonin. Several major families of serotonin receptors, each with several subtypes, are now recognized (Hoyer and Boddeke 1993; Hoyer et al. 1994). While the molecular cloning of these receptors proceeds rapidly, understanding the functional significance of many of the newly identified receptor subtypes in different types of aggression and anxiety remains a challenge at present (Wilkinson and Dourish 1991; Mos et al. 1992; Olivier et al. 1992; Barrett and Vanover 1993).

The preclinical study of the role of 5-HT receptor subtypes in different types of aggressive behavior is at an early stage. This is surprising since the 5-HT deficiency hypothesis of violent and aggressive behavior has been reiterated during the past 2 decades with vigor and enthusiasm. Ever since Brodie and Shore (1957) proposed brain 5-HT to subserve an inhibitory role in behavior and autonomic nervous activity, Hess' tropotrophic system appeared to have a neurochemical basis (Hess 1954). In fact, the tropotrophic role of serotonin continues to be the guiding principle for much preclinical and clinical aggression research. During the 1960s and 1970s, preclinical studies of fighting in singly housed mice and in mouse-killing rats associated low levels of brain 5-HT metabolites with a high incidence of fighting and killing (e.g., Garattini et al. 1967; Giacalone et al. 1968; Valzelli and Garattini 1968; Valzelli and Bernasconi 1979; Valzelli 1982;

Pucilowski and Kostowski 1983; Valzelli and Galateo 1984). Evidence from studies with highly aggressive humans with various antisocial, alcoholic or other diagnoses showed often an association with low 5-HIAA levels in spinal CSF (Brown et al. 1979; Linnoila et al. 1983; Virkkunen et al. 1989).

These correlational studies between a single value of 5-HT or its metabolite level in whole brain or cortical regions or CSF from spinal taps on the one hand, and a characteristic trait of excessive aggressive behavior on the other, remain inconclusive as to the causal relationship between these neurochemical and behavioral variables. Single measures of blood platelet or CSF 5-HIAA are difficult to relate to regulatory events at pre- and postsynaptic sites of anatomically discrete 5-HT neurons (Miczek et al. 1994b).

The development of more selective agonists and antagonists at the 5-HT receptor subtypes that act at somatodendritic, presynaptic autoreceptors or postsynaptic sites, associated with ion channel proteins or G proteins, is prompting research on their respective role in dominance, territorial and maternal aggressive behavior as well as defensive responses (Miczek et al. 1989; Olivier et al. 1989; Mos et al. 1992). Table 2 summarizes the key findings of drugs acting on 5-HT_1 receptors on various aggressive behavior patterns in comparison with those of benzodiazepines.

In confrontations with an intruder, resident male and female rats and mice engage in less frequent aggressive behavior after treatment with full agonists at 5-HT_{1A} receptors, such as 8-OH-DPAT or flesinoxan, and partial agonists, such as buspirone, ipsapirone and gepirone (Oliver et al. 1990; Miczek and Haney, unpublished observations).

By far the most promising evidence on the role of 5-HT receptors in aggressive behavior has been collected with experimental manipulations that target the 5-$HT_{1A,B}$ receptors. Highly effective and behaviorally specific reductions in a range of aggressive behavior patterns have been achieved with piperazine derivatives that act as mixed agonists at 5-HT_{1B} and 5-HT_{1A} receptors. Olivier and associates have summarized the evidence that eltoprazine and the more 5-HT_{1B} selective agonist TFMPP (*m*-trifluoromethylphenyl-piperazine) which also causes 5-HT release, decrease a resident rat's or mouse's attacks and threats toward an intruder, decrease a lactating female rat's attack toward an unfamiliar male approaching the nest, increase the electrical current threshold for evoking attack leaps and bites by hypothalamic stimulation in rats toward a stimulus rat, and reduce the incidence of biting during the formation of dominance hierarchies among young pigs (Olivier et al. 1990). The potent anti-aggressive effects across a range of animal species and situations is promising, although these "serenic" drugs may also increase responses to aversive events (Rodgers et al. 1992).

When the 5-HT_{1B} receptor is "knocked out" in mutant male mice, their frequency of attack behavior is more than double that of heterozygous mice (Saudou et al. 1994). Interestingly, no unusual changes in reproductive or feeding behavior, pain responses or sleep-waking activity are seen in the 5-HT_{1B} "knock-out" mice pointing to a relatively selective change in aggressive behavior. However, eltoprazine remains effective in decreasing aggressive behavior of these 5-HT_{1B} mutant mice suggesting that the 5-HT_{1A} receptor continues to modulate this behavior (Hen et al. 1994).

So far, the most specific modulations of aggressive behavior appear to be achieved via manipulations of the 5-HT_1 receptor family, whereas 5-HT_2 and 5-HT_3 antagonists suppress aggressive behavior with limited specificity. Remarkably, no 5-HT agonist or antagonist, acting pre-or post-synaptically, has been found to increase any type of aggressive behavior in animals.

From a pharmacological viewpoint, the current characterization of the various 5-HT receptor subtypes in anxiety and aggression is tantalizing and frustrating. For example, which are the requisite mechanistic changes during the period of chronic treatment with partial 5-HT_{1A} agonists that are necessary for clinical effects to emerge? Pharmacologically, adequate "silent" antagonists only now are becoming available in order to establish the 5-HT_{1A} receptor as the important site of action for buspirone, ipsapirone or gepirone (e.g., Hoyer and Boddeke 1993; Hoyer et al. 1994; Fletcher et al. 1993) Similarly, the pharmacological tools for study of the 5-$HT_{1B/D}$ and 5-HT_3 receptors remain too limited in order to adequately characterize the role of these receptors in experimental protocols for the study of anxiety and aggressive behavior.

Anxiolytics and affective vocalizations during aggression and anxiety

A seemingly insurmountable challenge to preclinical research on anxiolytics is the experimental quantification of the affective or emotional dimension of anxiety and aggression. The premise for this quest may be traced to Darwin's argument that emotions have evolved in species other than humans. As a matter of fact, the neurobiological mechanisms for emotional behavior have been studied in a range of mammalian species, including non-human primates (MacLean 1949; Skolnick and Paul 1982; Ploog 1989). Yet, preclinical psychopharmacological research on aggression and anxiety in the behavior-analytic tradition has largely circumvented the direct study of emotional behavior, by inferring emotional processes from disrupted ongoing behavioral performance (e.g., Estes and Skinner 1941; Brady 1956).

As summarized above, animal models are validated pharmacologically by their sensitivity to produce behavioral changes as a result of specific treatments rather than attempting to model specific changes in affect, i.e. pharmacological versus external validity. It

is important to recall that the critical defining features of many affective disorders, including anxiety, are their *emotional* nature and disruption of normal social functioning. Modeling specific affective expressions such as "fear" or "contentment" under socially relevant conditions may provide valuable insight in the neurobiology of anxiety that have not been adequately addressed in animal models (Mark and Ervin 1970; Redmond and Huang 1979; Winslow and Insel 1991b). A common characteristic of intense excitation when in pain or fear, is the emission of high-frequency calls. Both in mammals and in birds, the average frequency increases and the band of frequencies of individual vocalizations widens during fearful situations (Scherer 1986; Aubin and Bremond 1992).

Vocalizations in mammals are the product of respiratory, laryngeal and articulatory movements that are the result of activity of cranial and spinal motor neurons in the brain stem that in turn receive modulatory input from higher cerebral levels (Jürgens and Ploog 1981). For example, anatomical studies in squirrel monkeys identify the anterior cingulate cortex, the basal amygdaloid nucleus, dorsomedial and lateral hypothalamus, and midline thalamus in addition to the caudal periaqueductal grey area as the most prominent structures that yield vocalizations when electrically stimulated. Also, in rats, electrical stimulation of thalamic, hypothalamic and mesencephalic as well as medullary structures evokes ultrasonic vocalizations in the 20–30 kHz range (Yajima et al. 1980, 1981). Among the neural structures that are most effective for evoking vocalizations are those where $GABA_A$-benzodiazepine receptor complexes and 5-HT receptors are localized (Young and Kuhar 1980; Zifa and Fillion 1992). Whether modulation of vocalizations by anxiolytic drugs reflects an alteration in affective processes or is a modification of activity in laryngeal or respiratory activity remains a critical question.

Before highlighting the significance of vocalizations in socially arousing situations, it may be useful to consider recent evidence on attempting to quantify the *emotional* component of behavior in response to painful and startling stimuli.

Vocalizations in the context of pain and startle

Preclinical methods in pain research include those that evoke vocalizations by rats; these sounds are typically in the hearing range of the human experimenter (e.g., Hammond 1989). The stimulus-evoked audible vocalizations are sensitive to opioids' analgesic effects (Levine et al. 1984), and when withdrawn from chronic opiate treatment, rats "scream on touch" (Bläsig et al. 1973). Additionally, rats emit audible vocalizations after the painful electrical stimulus has terminated; these so-called vocalization afterdischarges are sensitive to opiates and benzodiazepine anxiolytics, and they may detect affective aspects of pain (Levine et al. 1984; Borszcz et al. 1994). Yet, the rat's audible squeal actually represents only a small fraction of its vocal responses immediately before, in reaction to a painful stimulus or after the termination of the stimulus. Additionally, single or bursts of ultrasonic vocalizations, in anticipation or in reaction to painful stimuli, are emitted in the 20- to 30-kHz frequency range with little frequency modulation and are the prevailing vocal expressions (Tonoue et al. 1986; Van der Poel et al. 1989; Van der Poel and Miczek 1991; De Vry et al. 1993; Sanchez 1993; Molewijk et al. 1995).

Opioid peptides, such as β-endorphin, met- and leu-enkephalin, and dynorphin proved effective in reducing pain-induced ultrasonic vocalizations, but audible squeals in reaction to the electric shock pulse remained unaffected (Tonoue et al. 1986). The ultrasonic vocalizations in the "anticipatory" phase, before the delivery of electric pulses to the tail, were particularly sensitive to morphine's suppressive effects, which were reversed by naloxone (Van der Poel et al. 1989). The environmental context that is associated with past electric shock deliveries is sufficient to induce ultrasonic vocalizations, and these calls may be interpreted as affective expressions in anticipation of pain (Miczek et al. 1991b). Opioid modulation of ultrasonic vocalizations by rats that anticipate the delivery of a painful stimulus may in fact point to a significant role of opioid peptides as well as *mu* and *delta* receptors in affective responses. Further support for a significant role of opioid peptides in the modulation of affective responses may be adduced from the effects of specific opioid receptor subtype agonists on vocalizations of rats that have been startled by an acoustic or tactile stimulus. *Mu* and *delta* selective receptor agonists, such as DAGO and DPDPE, potently inhibit 20- to 32-kHz ultrasounds in a naltrexone-reversible manner in male intruder rats (Vivian and Miczek, in preparation). These observations implicate *mu* and *delta* receptors in vocalization that may reflect affective distress.

Consistent with this hypothesis is the observation that rats that are withdrawn from opiate treatment emit ultrasonic vocalizations (Vivian and Miczek 1991). In addition to the well-known "scream-on-touch" and other prominent signs of autonomic distress, morphine-withdrawn rats emit ultrasonic calls in the 20- to 30-kHz as well as the 40- to 60-kHz range for a longer period of time than the somatic and autonomic withdrawal symptoms. These ultrasounds are particularly prominent when the morphine-withdrawn rat is challenged in a social situation or by a startling stimulus. Withdrawal from 30- or 60-day drinking of cocaine solution is also characterized by the emission of high rates of ultrasounds. This effect is most prominent during the first week after cocaine termination when the rats are challenged by tactile startle stimuli (Barros and Miczek 1994).

Evidence from recent studies with anxiolytic drugs provides important pharmacological validation for the contention that ultrasonic vocalizations may represent affective expressions by rats that are exposed to startling or painful stimuli. Anxiolytics that target especially 5-HT_{1A} and benzodiazepine-$GABA_A$ receptors effectively decrease the rate and duration of ultrasounds in those rats that emit these 20- to 30-kHz calls, when exposed to acoustic startle stimuli (Kaltwasser 1991) or to foot shock, but not tail shock (Cuomo et al. 1988; Rowan et al. 1990; De Vry et al. 1993; Sanchez 1993; Cullen and Rowan 1994; but see Van der Poel et al. 1989). No tolerance to the suppression of shock-elicited ultrasounds was seen with repeated administration of ipsapirone, and no rebound increase in calls was evident when ipsapirone administrations were terminated (De Vry and Schreiber 1993). One day after the last of ten diazepam administrations rats emit increased ultrasonic vocalizations, when prompted by acoustic startle stimuli (Miczek and Vivian 1993). Gepirone and diazepam reversed the increased ultrasound emission during diazepam withdrawal (Vivian et al. 1994a). It is, however, noteworthy that buspirone has not been shown to be effective in the clinical management of benzodiazepine withdrawal (Schweizer and Rickels 1986). When exposed to the environment where rats have received electric foot shock previously, they emitted fewer ultrasounds when given drugs with anti-panic activity such as alprazolam and 5-HT uptake blockers (Molewijk et al. 1995).

Evidence from comparative, chronic, withdrawal and antagonism studies has begun to pharmacologically characterize the vocalizations by rats exposed to situations where painful and startling stimuli are likely to occur. Potent and efficacious modulation of ultrasonic vocalizations and certain audible vocalization after discharges in rats appear to detect the selective effects of drugs acting on opiate, 5-HT_{1A} and benzodiazepine receptors that may play a role in affective expressions.

Vocalizations during social separation

Separation from a major attachment figure is considered as a primary cause of childhood separation anxiety, anticipatory anxiety and over-anxiousness as well as for generalized anxiety and panic disorders in adults (DSM IV, American Psychiatric Association 1994). The etiology and pharmacological modulation of separation distress in primates and other animals may be analogous to that observed in humans (Panksepp et al. 1978; Reite et al. 1981).

Primates

Vocalizations during social separation (i.e. "isolation calls") have been characterized pharmacologically more than other calls (Harris and Newman 1988; Newman 1988a; Kalin and Shelton 1989). Infant and juvenile primates separated from their mother or social group immediately respond with increased vocalizations, agitation, and motor activity (Reite et al. 1981; Wiener et al. 1988; Suomi 1991), as well as increases in heart rate, body temperature, and circulating cortisol levels and contact with the mother attenuates these changes (Mendoza et al. 1978; Coe et al. 1985). As social separation is prolonged, "protest" behaviors diminish and infants display "despair" (Suomi 1991). During the despair phase, infants typically display fewer vocalizations, a slouched posture, more self directed behaviors, less motor activity and fewer social interactions.

In infant rhesus monkeys, separation-induced "distress" vocalizations, defensive behaviors (e.g., freezing and crouching) and elevations in plasma ACTH and cortisol levels were reduced by diazepam (Kalin et al. 1987). Flumazenil blocked the decreases in plasma ACTH and cortisol produced by diazepam, but not the reductions in vocalizations. Specifically, defensive "bark" calls were significantly decreased by diazepam, and unaffected by morphine or naloxone, whereas the affiliative "coo" calls were reduced by morphine and increased by naloxone but are unaltered by diazepam (Kalin et al. 1988; Kalin and Shelton 1989).

Socially separated infant and adult squirrel monkeys generally emit isolation "peeps" and show increased adrenal corticosteroid levels (Winter 1968; Symmes et al. 1979). In young animals these calls may solicit social contact with mother and group members (Newman 1988b), and *tactile* contact with the mother significantly reduces adrenal and vocal "distress" responses (Mendoza et al. 1978; Wiener et al. 1988; Weerts and Miczek, submitted). Recently, behavioral "protest" in reaction to social separation in juvenile squirrel monkeys was confirmed by characteristically high rates of "peep" vocalizations and explosive motor activity (Fig. 1; Weerts and Miczek, submitted). These vocalizations were hypothesized to be affective expressions of anxiety-like states produced by separation from the mother and familiar group members. Both vocalizations and explosive motor behaviors were highly sensitive to full and partial benzodiazepine receptor agonists and to antagonists. The full agonist, chlordiazepoxide, as well as the antagonist, flumazenil, dose-dependently reduced calls and explosive motor behaviors (e.g. rapid locomotion, jumping, leaping at and shaking the cage; Fig. 1). In contrast, the partial agonist bretazenil, which may be less sedative at anxiolytic doses (Haefely et al. 1990), reduced calls only at very high doses. In addition, alcohol did not attenuate vocalizations, except at an ataxic dose. The potent anxiolytic-like effects of flumazenil on squirrel monkey "peeps" were closely similar to effects reported for isolated rat pup vocalizations (Insel et al. 1986; vide infra), although this effect is not always as significant as in

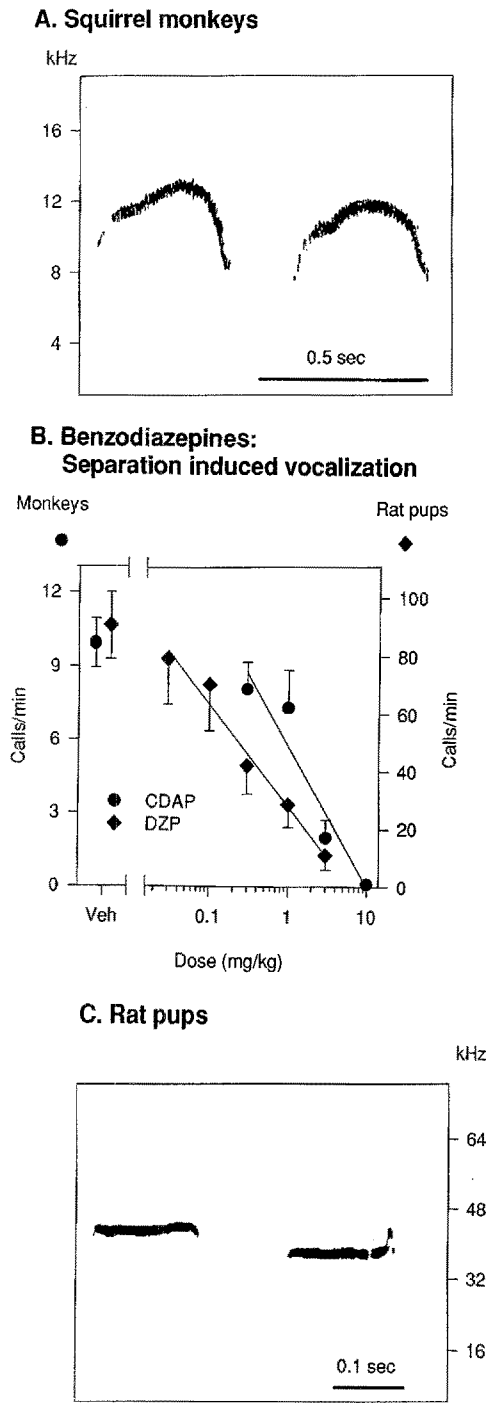

Fig. 1A Sonogram of "peep" vocalizations emitted by a juvenile squirrel monkey when separated from the native social group. **B** Effects of chlordiazepoxide on "peep" vocalizations by juvenile squirrel monkeys, and of diazepam on ultrasonic vocalization by rat pups. The data are expressed as a function of dose. **C** Sonogram of ultrasonic vocalizations emitted by a rat pup when separated from the littermates and dam. (Data from Weerts and Miczek, submitted, and from Vivian et al., submitted)

juvenile rhesus monkeys' "distress" vocalizations (Kalin et al. 1987).

Flumazenil may interact with endogenous substances and exert different effects depending on the affective state of the animal, as suggested for separated rat pups (Insel et al. 1989). Although attractive, the evidence for an endogenous ligand remains to be substantiated. Flumazenil's effects in preclinical procedures have been interpreted as both "*anxiolytic*" (e.g., increased social interactions, exploratory behaviors and feeding) and "*anxiogenic*" (e.g., reduced social behaviors, partial generalization to PTZ) depending on the dose and testing situation (File and Pellow 1986; Haefely 1988; Weerts et al. 1993a). Also, squirrel monkeys may be unusually sensitive to the partial agonist actions of flumazenil. Previously, squirrel monkeys were found to be more sensitive to benzodiazepine receptor antagonists such as flumazenil and ZK 93426 and partial inverse agonist such as Ro 15-4513 than rodents. Flumazenil's hyperphagic effects as well as the proconvulsant effects of Ro 15-4513 were readily detected in squirrel monkeys, but not in rats (Weerts et al. 1993a).

In selected adult squirrel monkeys, isolation "peep" calls were sensitive to opiate modulation; morphine suppressed isolation calls, whereas naloxone increased isolation calls and blocked the reductions in calls produced by morphine (Newman 1988a). In addition, "peeps" were also suppressed by imipramine and clonidine, when tested 1 h after administration (Harris and Newman 1988). Clonidine-induced suppression of isolation "peeps" were reversed by yohimbine, but not by prazosin. However, yohimbine increased isolation "peeps" when administered alone (Harris and Newman 1987). In addition to benzodiazepine anxiolytics, opioid and adrenergic systems also appear to modulate isolation-induced vocalizations in adult squirrel monkeys.

Young rodents

When separated from the litter and mother and when exposed to cold or rough handling, neonatal rats and mice emit ultrasonic vocalizations as pure tone whistles, ranging in frequency from 20- to 60-kHz, and 0.1- to 3.5-s in duration (Fig. 1C). Benzodiazepine anxiolytics, such as chlordiazepoxide and diazepam, decreased pup ultrasounds without altering locomotor behavior (Gardner 1985), while inverse agonists, such as DMCM, FG 7142 and pentylenetetrazol, increased ultrasounds (Insel et al. 1986; Gardner and Budhram 1987; Nastiti et al. 1991). Flumazenil reversed the effects of agonists and inverse agonists implicating the benzodiazepine receptor in the mediation of pup ultrasounds. Furthermore, socially isolated pups show decreased flumazenil binding in limbic structures (Insel 1989).

GABA agonists such as muscimol and baclofen were found to decrease ultrasounds in mouse pups (Nastiti et al. 1991), and recently the role of the benzodiazepine-$GABA_A$ receptor complex in rat pup ultrasonic vocalization was more fully characterized (Fig. 1; Vivian et al., submitted). Allopregnanolone, a neurosteroid

Table 3 Vocalizations during aggression and anxiety -prototypic effects of anxiolytics. *AV* Audible vocalizations, *USV* Ultrasonic vocalizations, High *USV* 31–70 kHz, Low USV 20–30 kHz, *CDP* chlordiazepoxide, *DZP* diazepam, *FNP* flunitrazepam, *BUS* Buspirone, *GEP* Gepirone; *IPS* ipsapirone, 8-*OH-DPAT* 8-OH-dipropylaminotetralin. All doses refer to intraperitoneal administration, unless stated: (IM) intramuscular route

Method	Benzodiazepine anxiolytics				5-HT agonists			
	Drug	Dose (mg/kg)	Effect	Reference	Drug	Dose (mg/kg)	Effect	Reference
Aversive environmental stimuli (adult rats)								
Footshock	DZP	0.5–1	↓	Cuomo (1988)	BUS	0.3–10	↓	De Vry et al. (1993)
Low USV					GEP	1–5	↓	Cullen and Rowan (1994)
					8-OH-DPAT	0.06–0.24		Sanchez (1993)
Startle	FNP	0.5	↓	Kaltwasser (1991)	IPS	5	↓	Kaltwasser (1991)
Low USV	DZP	1–3	↓	Vivian et al. (1994a)	GEP	0.6–1	↓	Vivian et al. (1994a)
Social separation								
AV Primates	DZP	1	↓	Kalin et al. (1987)				
	CDP	3–10 (IM)	↓	Weerts and Miczek, submitted				
USV Rat pups	DZP	2.5–10		Mos and Olivier (1989)	BUS	1–3	↓	Mos and Olivier (1989)
					8-OH-DPAT	0.1–0.2	↓	
Aggression								
AV Primates	CDP	0.3(IM)	↑	Weerts and Miczek, submitted				
Response to threat of aggression (adult rats)								
a) "anticipatory"								
High USV	DZP	6–10	↓	Tornatzky and Miczek (1995)	GEP	0.3–6	0	Tornatzky and Miczek (1995)
Low USV	DZP	1–10	0		GEP	3–6	↓	
b) "reactive"								
High USV	DZP	10	0	Tornatzky and Miczek (1995)	GEP	0.3–6	↓	Tornatzky and Miczek (1995)
Low USV	DZP	1–10	0	Vivian and Miczek (1993a)	GEP	3–6	↓	Vivian and Miczek (1993a)

acting on this receptor complex, proved active in suppressing pup ultrasounds (Zimmerberg et al. 1994). Using the suppression of pup ultrasounds as a functional endpoint, the order of relative potency of agents acting on this receptor complex was muscimol > alprazolam > diazepam > pentobarbital = allopregnanolone > ethanol (Vivian, Barros and Miczek, submitted). The effects of diazepam and alprazolam were reversibly antagonized by flumazenil, and those of muscimol by biccuculine confirming the benzodiazepine and $GABA_A$ receptors as relevant for the suppression of pup ultrasounds. Allopregnanolone potentiated the suppressive effects of diazepam and alprazolam markedly, and those of pentobarbital moderately. Conversely, none of the antagonists of this receptor complex (i.e. flumazenil, bicuculline, and picrotoxin) altered the effects of allopregnanolone on pup ultrasounds.

The benzodiazepine-$GABA_A$ receptor complex contains receptor and recognition sites for chemically diverse agents, extending most recently to neurosteroids, that are significant targets for modulating pup ultrasounds. It will be essential to decipher the necessary molecular configuration of novel substances with anxiolytic-like effects on these distress signals.

The 5-HT_{1A} agonists 8-OH-DPAT, flesinoxan, and the partial agonists gepirone, ipsapirone and buspirone, the 5-HT_2 agonist DOI as well as the 5-HT uptake blockers such as fluvoxamine, zimelidine, and clomipramine proved effective in suppressing pup ultrasounds (Hard and Engel 1988; Mos and Olivier 1989; Winslow and Insel 1990a, b). Interestingly, agonists at the 5-HT_{1B} receptor TFMPP and CGS12066B increase the rate of pup ultrasounds. The present evidence points to 5-HT_{1A} receptors as being significant targets for modulating pup ultrasounds. However, in order to implicate the 5-HT_{1A} receptors in a selective suppression of pup ultrasounds it will be important to reverse agonist effects.

Separation-induced vocalizations were initially linked to opioid peptides and their receptors (Panksepp et al. 1978). Rat pup ultrasounds are suppressed by morphine and by selective *mu* and *delta* receptor agonists such as DAGO and DPDPE, while the *kappa* receptor selective agonist U50488H increased these vocalizations (Carden et al. 1990, 1994; Kehoe and Shoemaker 1991; Barr et al. 1994; but see Winslow and Insel 1991a). The opposite effects of *mu* and *delta* receptor agonists versus *kappa* receptor agonist parallels the putative reinforcing and aversive effects of these drugs and are consistent with their proposed role in affective behavior.

Vocalizations during threatening situations

Rodents and primates emit a range of loud and frequent vocalizations in response to the threats and

attacks of an aggressive opponent and as anti-predator signals. Functionally, these calls may represent affective expressions, but they also may provide information about the sender, are part of defensive and submissive displays, and may warn conspecifics about imminent danger. Recent evidence indicates that these calls can be modified by mood-altering drugs, particularly benzodiazepine and 5-HT drugs with anxiolytic and potentially antidepressant effects.

Vocal responses to threats and attacks

As many currently available anxiolytics are chiefly effective in anticipatory or generalized anxiety disorders (Molander 1982), it is not surprising that the most successful preclinical tests involve animals who anticipate fear-provoking events as a result of conditioning (e.g., Estes and Skinner 1941). When a rat enters a locale that signals the presence of a potential attacking opponent, it begins to emit bursts of ultrasonic vocalizations in the 20–30 as well as 40–60 kHz range (Tornatzky and Miczek 1994, 1995).

Diazepam and alcohol effectively attenuated high-frequency ultrasounds by intruder rats when they were exposed to the environment where they were previously attacked, while gepirone suppressed low-frequency ultrasounds (Tornatzky and Miczek 1995). In this early, anticipatory phase of a social confrontation, the emission of ultrasounds is accompanied by tachycardia and hyperthermia, all of which were reduced by alcohol, diazepam and gepirone. These observations point to the phase preceding a social confrontation as engendering a constellation of behavioral acts, including vocal responses, and physiological changes that are particularly sensitive to the effects of drugs with an anxiolytic-like profile, albeit each with a different mechanism of action.

In fact, the marked behavioral and physiological activation in reaction to attacks by an aggressive opponent remains unaffected by anxiolytics in both experienced and inexperienced intruder rats (Vivian and Miczek 1993a; Tornatzky and Miczek 1995). During the reactive phase of a social confrontation, intruder rats' cardiovascular and thermoregulatory functions are maximally activated and upright and supine postures are accompanied by bursts of loud and frequent ultrasonic vocalizations. Even at sedative and muscle-relaxant doses, neither diazepam, alcohol nor gepirone affected the emission of audible and low-frequency ultrasonic vocalizations. It appears that drugs that are clinically effective in ameliorating generalized anxiety are ineffective in modulating vocalizations under conditions of high-intensity confrontations. It is noteworthy that morphine and the *mu* and *delta* receptor-selective agonists, DAGO and DPDPE decreased ultrasounds by male or female intruders while they were being attacked (Vivian and Miczek 1993b; Haney and Miczek 1994, submitted).

When shielded from physical harm, intruder rats continue to emit low- and high-frequency ultrasonic vocalizations in response to the threats of an aggressive opponent. The intensity of a confrontation that relies upon postural displays and vocal responses is considerably lower than that during the actual fight as indicated by the magnitude of endocrine, cardiovascular and thermoregulatory responses (Miczek et al. 1991a). Under these threatening conditions gepirone decreased low-frequency ultrasounds in experienced and inexperienced intruder rats (Vivian and Miczek 1993a; Tornatzky and Miczek 1995). Diazepam attenuated the emission of high-frequency ultrasounds in animals that were threatened for the first time. Interestingly, drugs such as opiates and clonidine that have anxiolytic activity in some individuals, also were effective in reducing high-frequency ultrasounds in this phase of the social confrontation (Vivian and Miczek 1993b; Haney and Miczek 1994; Tornatzky and Miczek 1994). However, the suppression of high-frequency ultrasounds may be part of the sedative effects of diazepam, morphine and clonidine, since these effects are seen only at higher doses.

A most intriguing pharmacological profile of effects on ultrasonic vocalizations during social confrontations is emerging. Drugs with known or putative anxiolytic activity attenuate ultrasonic vocalizations in animals that anticipate a confrontation with an aggressive opponent, but not in those who actually defend and submit in an intense fight. This distinction between anticipatory and reactive phases of an encounter offers the opportunity to differentiate between drug effects that may be relevant to the generalized anxiety disorder vs those that may correspond to panic states.

Anti-predatory vocalizations

Under field conditions, squirrel monkeys and vervet monkeys confronting aerial and carnivorous predators emit vocalizations with distinct structural elements and in specific frequency ranges (Struhsaker 1967; Seyfarth et al. 1980; Newman et al. 1983; Marler 1982). Benactyzine, an anticholinergic drug, prompted adult squirrel monkeys to emit higher rates of alarm calls in response to threatening objects presented by the experimenter, but not to increase isolation calls (Glowa and Newman 1986; Glowa et al. 1988). This effect was blocked by the cholinesterase inhibitor, physostigmine, indicating pharmacological specificity to cholinergic mechanisms. By contrast, yohimbine and naloxone increased squirrel monkey isolation calls out did not alter alarm calls (Harris and Newman 1988). Vocalizations elicited during situations of isolation and alarm can be pharmacologically dissociated.

Whether or not antipredator alarm calls are modified by anxiolytic or antidepressant drugs, and whether these effects are selective, has begun to be addressed in confrontations between a cat and rats. When presented with a potential predator, a cat, male and particularly female rats emit ultrasonic vocalizations, when in the presence of other colony members (Blanchard et al. 1990, 1991, 1992). Morphine, at an analgesic dose, suppressed these antipredator vocalizations, but even sedative doses of alcohol and diazepam left these calls unchanged (Blanchard et al. 1990; Shepherd et al. 1992; Blanchard, unpublished observations). It appears that the antipredator vocalizations are important in the survival of the species, assuming a functional significance beyond expressions of "affect" or "distress", as these calls remain intact even in profoundly sedated individuals.

Vocalizations during aggressive confrontations

Vocalizations may represent signals that are critically significant to sender as well as receiver, in addition to being affective expressions. Successful communication of information during situations of conflict increases the survival of individuals as well as increasing the evolutionary fitness of the species (Smith 1985). For example, when confronting a rival, and prior to physical attack, dominant squirrel monkeys emit vocalizations such as cackle and harsh display calls (Jürgens 1979). It is possible to evoke these types of calls (and others) by activating glutamate receptors or by blocking $GABA_A$ receptors in the periaqueductal grey region of squirrel monkeys, even in the absence of social provocation (Jürgens and Lu 1993; Lu and Jürgens 1993).

Drugs acting on the benzodiazepine-$GABA_A$ receptor complex may interfere with the production and perception of social signals that communicate subordination or appeasement during social confrontations (e.g., Miczek et al. 1994). Initiation of aggressive behaviors can be enhanced in alcohol-treated dominant male monkeys, whereas subordinates treated with alcohol are more often the *recipient* of aggressive interactions, presumably due to inappropriate social signalling (Miczek et al. 1984; Winslow and Miczek 1985).

When adult male squirrel monkeys confront an unfamiliar male rival under experimental conditions, they display various visual and vocal threats (Weerts and Miczek, submitted), closely similar to those observed in the field (Newman 1985) as well as to those elicited by electrical brain stimulation of limbic structures (Jürgens 1988). Vocalizations that accompany aggressive displays are "noisier", and calls with wider band frequency ranges have been interpreted to reflect greater levels of arousal and aversion (Jürgens 1982), as well as "distress" and "maximum intensity" aggressive displays (Newman 1985). Dominant squirrel monkeys' threat vocalizations, especially peeps, peaked during the first minute of aggressive encounters and declined during the course of the confrontation (Weerts and Miczek, submitted), as this habituation is characteristic for aggressive interactions in other species (e.g., Winslow and Miczek 1984; Miczek et al. 1992). However, neither chlordiazepoxide nor alcohol "disinhibited" vocal behavior that was suppressed in habituated opponents. Chlordiazepoxide and alcohol substantially increased threat peeps during the first minute of aggressive interactions when they were most frequent. Aggressive displays and threat peeps were maintained across a wide range of chlordiazepoxide doses, even though the highest dose increased inactivity and produced ataxia.

By contrast, vocalizations during feeding and affiliative behavior include peeps, twitter, chuck and cackle calls directed at familiar group members (Weerts, Macey and Miczek, submitted). Under these conditions, peep vocalizations were not altered by benzodiazepines including chlordiazepoxide. It is evident that similarly structured calls are differentially altered by anxiolytic drugs depending on the social contexts. These observations suggest that situational influences such as provocation and interaction with other individuals within the social context are critical determinants of benzodiazepine and alcohol effects on vocalizations and social behavior.

The production and structure of vocalizations as well as subsequent behavioral responses are indeed *context specific*. In pigtailed macaques and rhesus monkeys, victims of aggression elicit structurally distinct vocalizations when attacked by a dominant vs. a subordinate (Gouzoules et al. 1984), "recruiting" aide in a rank-dependent manner from other group members or kin. Playback studies indicate that vocalizations in the absence of the appropriate contextual cues fail to elicit predictable behavioral responses (Gouzoules et al. 1984; Biben and Symmes 1991).

Conclusions

Preclinical research on drugs that modulate normal and pathological affective processes can derive considerable benefits from the study of vocalizations in animals. The validity of vocalizations as a focus for the study of anxiolytic and other mood-altering drugs derives primarily from their occurrence in intense phases of reproduction, maternal separation, social conflict and confrontations with predators and distressing events. It has become apparent under which experimental conditions it is possible to engender different types of loud and frequent vocalizations in laboratory rodents and primates that correspond to those under field conditions. The methodological requirements for their accurate recording and quantitative analysis have become adequately established. In well-defined contexts, typically including an audience, certain vocalizations may represent affective expressions which renders them

particularly attractive for research with anxiolytic and other mood-altering drugs. Pharmacological validity has become apparent when, for example, benzodiazepines selectively attenuate a rat's ultrasonic vocalization in an antagonist-reversible manner. Drugs acting on 5-HT receptors, particularly on the 1A and 1B/D subtypes effectively suppress vocalizations by maternally separated pups or by adults rats that anticipate a social confrontation or aversive environmental event. It appears quite feasible that experimental conditions can be developed prompting vocal expressions that are sensitive to drugs effective in the treatment of affective disturbances ranging from panic to depression. In addition to communicating affective expressions, vocalizations may also represent important messages that inform the receiver about critical features of the sender. Many psychotropic drugs such as alcohol distort this communication and thereby disrupt the patterns of social and aggressive behavior.

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