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The Mouse Defense Test Battery: pharmacological and behavioral assays for anxiety and panic

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Abstract

The Mouse Defense Test Battery was developed from tests of defensive behaviors in rats, reflecting earlier studies of both acute and chronic responses of laboratory and wild rodents to threatening stimuli and situations. It measures flight, freezing, defensive threat and attack, and risk assessment in response to an unconditioned predator stimulus, as well as pretest activity and postthreat (conditioned) defensiveness to the test context. Factor analyses of these indicate four factors relating to cognitive and emotional aspects of defense, flight, and defensiveness to the test context. In the Mouse Defense Test Battery, $GABA_A$–benzodiazepine anxiolytics produce consistent reductions in defensive threat/attack and risk assessment, while panicolytic and panicogenic drugs selectively reduce and enhance, respectively, flight. Effects of $GABA_A$–benzodiazepine, serotonin, and neuropeptide ligands in the Mouse Defense Test Battery are reviewed. This review suggests that the Mouse Defense Test Battery is a sensitive and appropriate tool for preclinical evaluation of drugs potentially effective against defense-related disorders such as anxiety and panic.

Keywords: Mouse Defense Test Battery; Anxiety; Panic; $GABA_A$–benzodiazepine receptor; 5-HT (5-hydroxytryptamine, serotonin); Neuropeptide

1. Defensive behaviors

1.1. Phenomenological status

The unconditioned defensive behaviors of rodents appear to consist of at least the following: flight, hiding, freezing, defensive threat, defensive attack, and risk assessment. Undoubtedly, more such defensive behaviors remain to be discovered or analyzed. These are species-typical (i.e. typically expressed by individuals of those species under appropriate circumstances) but not species-specific: they occur in much the same form across a variety of mammalian species (Blanchard et al., 2001). The statement that these are unconditioned reflects that each behavior can be elicited in wild rats or wild mice (and with partial exceptions to be noted later) in laboratory or domesticated strains of rats and mice without prior relevant experience (see Blanchard, 1997 for review of behavioral analyses).

In these animals, the occurrence of defensive behaviors is strongly associated with threatening events. Confrontation with a predator or unconditioned predator stimuli such as odors; salient and unexpected stimuli such as loud noises, sudden motion, or air puffs; and novel situations, high places, or moving substrate (e.g. Blanchard and Blanchard, 1972; Endler et al., 1986; King, 1999) can all elicit defense, as can stimuli or situations associated with pain. The relationship between defense-eliciting stimuli/situations and the particular defensive behaviors that are elicited is complex, but relatively clear. Manifest and tangible threat stimuli tend to elicit flight when an escape route is available, hiding if there is a place of protection or concealment and freezing when neither of these features is present. As these threats approach and contact the animal, they elicit first defensive threat (e.g. sonic vocalizations) and then defensive attack.

Threat stimuli may also elicit patterns of risk assessment. These may include orientation to the threat source, sensory

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scanning (sniffing and auditory and visual scanning, marked by side-to-side head sweeps), and approach/investigation. Both the motionless orientation phase and the approach phase of risk assessment are marked by low-back, stretched posture or movement, respectively, that appear to permit investigation of the threat source while minimizing the probability that the animal will be detected by the threat. In line with this view, movement during the "stretch-approach" phase of risk assessment is punctate, marked by periods of motionlessness interspersed with rapid, stealthy movement. Pinel and Mana (1989) have shown that this activity is associated with gathering of information about the threat source.

1.2. Evolutionary status

While defensive behaviors have not been so systematically investigated in nonrodent species, there are a number of studies or observations of specific defenses in other mammals as well as inframammalian species (Edmunds, 1974), supporting a view that the particular defensive behaviors discussed here are not "species-specific." Briefly, the most primitive mammals, the monotremes, display immobility and hiding (rolling into a ball, digging holes, and hiding there; echidnas), flight, and defensive threat and attack (platypuses). Marsupials may also show flight or freezing as well as defensive threat and attack, and these appear under circumstances similar to those that control these same behaviors in rats and mice. Such similarities in the descendants of animals (the ancestors of monotremes and marsupials) from which placental mammals had diverged by about 135 million years ago (Eisenberg, 1981) support an ethological concept of behavioral "homology" that provides a strong argument against the view that these core defensive behaviors are specific to any particular species.

Among placental mammals, habitat and food preference specializations have led to differential emphasis on particular defensive behaviors. Thus, for the most part, ungulates rely largely on flight as a defense (Edmunds, 1974), while cats are particularly noted for defensive threat (the familiar "Halloween cat" display) and a very potent form of defensive attack (Leyhausen, 1969). Nonetheless, both ungulates and cats show a full range of defenses. Similarly, while the concept of risk assessment is relatively new in the experimental animal behavior literature, a related set of behaviors, collectively labeled "vigilance," has long been used to describe an array of activities aimed at localizing and identifying threat in both birds and mammals (e.g. Treves, 1999).

Although evolutionary conservatism of a range of defensive behaviors contributes to the scientific importance of their investigation, in the specific context of pharmacology the most important is similarity of behaviors—and their biological mechanisms—between species in which a wide variety of experimental procedures are feasible, and humans. Systematic experimental studies of human defensive behaviors would involve a range of legal and ethical problems, and these studies have not yet been done. However, a recent attempt to determine "first-choice" defensive behaviors to threat stimuli and situations described in scenarios provides considerable support for a view that people utilize flight, hiding, freezing, defensive threat/attack, and risk assessment extensively in dealing with threat. Moreover, hypotheses concerning the relationship of threat stimulus/situational characteristics and specific defensive behaviors, based on rodent experiments, were strongly supported by this study of human response choices (Blanchard et al., 2001). While this study says nothing about the biological mechanisms involved, it does provide some assurance that despite the enhanced array of human social, verbal, and technological defenses, there remains considerable conservation of infrahuman mammalian defenses in the normal human defense repertory.

2. Development of the Mouse Defense Test Battery

2.1. The Fear/Defense Test Battery

The same research that described defensive behaviors in laboratory rodents also yielded the first test situations for analysis of drug effects on these same responses. In particular, a long (6 m), oval runway apparatus utilizing a human experimenter as the threat stimulus was developed in order to permit the adequate expression of flight behaviors in wild rats (*Rattus norvegicus*). These animals, both wild-trapped or first-generation laboratory-bred, show a very high probability of rapid flight to an oncoming human when flight is possible, but switch abruptly to freezing when the closure of a door changes the oval (endless) runway to a straight alley. The selectivity of this change is notable, from about 97% flight responses in the runway to virtually 100% freezing (at close distances) in the straight alley. As the threat approaches freezing. Increases up to about 1-m distance when defensive threat abruptly appears, followed by defensive (jump and biting) attack as contact between threat and subject becomes imminent.

Laboratory rats (Long–Evans strain) ran in this same test showed the same range of behaviors but with substantial differences in the magnitude of some of these. The most dramatically different behavior was defensive attack (biting), which declined from near maximal levels in wilds to zero in response to stimulus contact (touch). The statement that this had not totally disappeared in the lab rats is supported by a finding that 2% of these animals did attempt to bite when being picked up following the test. Flight was also considerably reduced (from 97%, averaged over a dozen studies of wild rats) to 57% in the Long–Evans laboratory rats.

These differences, in particular the virtual abolition of defensive threat/attack for laboratory rats, indicated the necessity of using wild rats should the test be employed for the purpose of evaluating potentially defense-reducing

manipulations. Thus, pharmacological studies using this task (Blanchard et al., 1988, 1989a,b, 1991b; Rodgers et al., 1990; Shepherd et al., 1993) routinely employed wild-trapped *R. norvegicus* and *Rattus rattus*. The task was labeled the Fear/Defense Test Battery to reflect that it involved those defensive behaviors (e.g. fear) that respond particularly to clearly present (manifest) and discrete threat stimuli; in contrast to threat that is anticipated, cued, or potential rather than present, also ambiguous or amorphous. This followed a well-established tradition in psychology (e.g. Freud, 1930; Estes and Skinner, 1941) of characterizing responses to the latter as anxiety.

These tests indicated that the benzodiazepine receptor agonists diazepam, chlordiazepoxide, and midazolam selectively reduced defensive threat vocalizations in wild rats with little additional effect (i.e. a reduction in flight with midazolam only, Blanchard et al., 1989a,b). Buspirone and gepirone, both 5-HT_{1A} agonists, similarly reduced defensive threat vocalizations or defensive attack without impacting flight, avoidance, or freezing (Blanchard et al., 1988, 1989b). However, in the first use of mouse subjects in this situation (and with the long rat runway/alley), the 5-HT_3 antagonist, ondansetron, had essentially no effect on any of these defense measures (Shepherd et al., 1993). Scopolamine hydrobromide and scopolamine methylbromide also produced no effect on rat defensive behavior in the Fear/Defense Test Battery (Rodgers et al., 1990).

2.2. The Anxiety/Defense Test Battery

The Anxiety/Defense Test Battery was designed specifically to include more ambiguous threat stimuli and to measure the defensive behaviors that selectively occur to these. That there are defensive behaviors different from flight, freezing, and defensive threat and attack had been made clear in studies of rats' responsivity to threat in a seminaturalistic situation, the Visible Burrow System (Blanchard and Blanchard, 1989). The Visible Burrow System is a large enclosure containing an open "surface" area as well as tunnels and chambers (burrows) designed to be similar to those that rats (wild or laboratory) construct in dirt substrate, when this is available. When mixed-sex groups are allowed to live in such a colony for several days, they develop a routine of activity and a male dominance hierarchy that have proved interesting for other purposes. However, when a cat is briefly placed in the "surface" area and then removed, the behaviors of the colony animals change dramatically and durably. They flee to the burrows and freeze there, later showing a pattern of risk assessment that involves orientation to the openings of the surface while in a stretched posture, followed by stretched approaches to the surface, and episodes of head-poking to scan and sniff the surface. These risk assessment activities and others (e.g. the corner run) that may be more specific to the situation occur over a period of 20 h or so after removal of the cat, and during this time, other activities such as sexual behavior appear to be suppressed (Blanchard and Blanchard, 1989).

These findings suggested that risk assessment is elicited specifically by potential threat stimuli. Logically, behavioral inhibition (reduction in normal activity) would be expected to occur whenever a strongly elicited behavior dominates; this includes responses to nonambiguous as well as ambiguous threat stimuli. However, in contrast to flight or freezing, both of which clearly preclude other activities while they occur, risk assessment may occur on a very intermittent basis over a long period of time such that one might expect it not to be incompatible with other behaviors. As the Visible Burrow System studies nonetheless indicated that risk assessment was associated with decreases in other activities, some internal inhibitory state appeared to be operative. Thus, both risk assessment and behavioral inhibition were incorporated into a rather loosely organized set of tasks collectively labeled the Anxiety/Defense Test Battery. Each of these included some type of ambiguously threatening stimulus; typically a cat odor, or even the cat itself, separated from the rat subject by a screen, making the cat's threat potential (if not the cat itself) somewhat ambiguous. In addition to risk assessment and inhibition of normal behaviors, freezing and proximic avoidance of such stimuli were possible. However, since the predator could not approach/contact the subject, no defensive threat or attack by the rat subject was ever seen in this situation.

When the classic benzodiazepine receptor agonist diazepam and chlordiazepoxide was administered in such situations (Blanchard et al., 1990a,b), they produced more effects than had been seen in the Fear/Defense Test Battery. Both of these drugs reduced avoidance of the threat stimulus, as well as behavioral inhibition. They also altered risk assessment, albeit in a manner that was initially very puzzling. When risk assessment was evaluated in the context of predator odors, its baseline magnitude was high and both of these benzodiazepine receptor agonists reduced it; conversely when the actual cat was present, risk assessment was low, and both benzodiazepine receptor agonists increased it. 5-HT_{1A} agonists gepirone and 8-OH-DPAT produced similar patterns, except that 8-OH-DPAT failed to reduce risk assessment to the cat odor. In contrast to the benzodiazepine receptor agonists, however, they also reduced freezing (Blanchard et al., 1992 and unpublished results).

On the basis of parallel effects in the Fear/Defense Test Battery and Anxiety/Defense Test Battery for the two benzodiazepine receptor agonists, both of them very commonly used anxiolytics, we suggested (Blanchard et al., 1993a) that risk assessment, defensive threat/attack, and behavioral inhibition were particularly sensitive to anxiolytic drugs. The substantial agreement with this profile of effects for 5-HT_{1A} agonists, some of which had been utilized clinically as anxiolytics (Jacobson et al., 1985), supported this view, as did findings that alcohol produced similar patterns of risk assessment and (at higher doses) reduced defensive threat/attack, albeit with a paradoxical low dose enhancement of the latter

(Blanchard et al., 1990a, 1992). Ritanserin, a 5-HT_{2A}/5-HT_{2C} receptor antagonist, reduced risk assessment in the cat odor situation but was without effect on other Anxiety/Defense Test Battery measures. It has been reported to be clinically effective against some (Eison and Eison, 1994) but not all (Den Boer et al., 1995) types of anxiety. Specificity of this profile of effects was further emphasized by findings that drugs such as the 5-HT_3 receptor antagonists MDL 72222 and ondansetron were without effect in these models (Shepherd et al., 1993). Although effective against chemotherapy-induced nausea and vomiting (Gregory and Ettinger, 1998), 5-HT_3 receptor antagonists appear to have little systematic effect on anxiety in clinical populations (Broocks et al., 1998). In addition, imipramine, which is effective against anxiety (e.g. McLeod et al., 2000; Russell et al., 2001), produced significant and parallel effects on three of the four Anxiety/Defense Test Battery measures shown to be systematically altered by the benzodiazepine and 5-HT_{1A} receptor agonists (Blanchard et al., 1993b). Morphine, a drug that had produced decreases in defensive threat vocalizations similar to those of the classic benzodiazepine receptor agonists, in the Fear/Defense Test Battery, tended in the Anxiety/Defense Test Battery to produce changes opposite to those of anxiolytics. This lack of agreement is compatible with failure to find a systematic effect of morphine on anxiety in human studies (e.g. Kay and Healy, 1984).

Although the bidirectional effect of anxiolytics on risk assessment to cat and cat-odor exposure was extremely consistent, it also presented an apparent paradox (Blanchard et al., 1991a). If risk assessment reflected anxiety, why did anxiolytic drugs increase this measure in situations involving exposure to a cat? The rat Visible Burrow System provided an important clue, in that active risk assessment behaviors oriented toward the open area in which the cat had been presented occurred only after a several-hour-long period in which the rats primarily displayed freezing in the depths of the tunnels. During this period, the nonappearance of the cat or any further indication of its presence presumably acted to reduce both the intensity and the certainty of threat. As threat intensity declined and threat ambiguity increased, the animals began to approach the surface area entryways to peer out and scan the surface and eventually to reenter it. However, after a long period of intermittent risk assessment of a genuinely nonthreatening situation (i.e. the cat is not present), rats gradually reduce their levels of risk assessment and return to normal activities. This pattern indicated that in rats, the onset of risk assessment from a baseline of freezing reflects reduced defensiveness. However, when risk assessment is itself the predominant ongoing behavior, decreased risk assessment is also associated with reduced defensiveness. This view is compatible with findings that risk assessment is associated with gathering of information about the threat source (Pinel and Mana, 1989) as well as analyses (Blanchard et al., 1991a) of the role of these activities in maintaining or reducing defensiveness, in accord with the information that these activities provide concerning threat or danger. From this perspective, reduced risk assessment and decreased behavioral inhibition (i.e. enhancement of normal activity in the presence of threat) are seen as different aspects of the same process, and the extent to which they occur together in response to drugs (e.g. diazepam, chlordiazepoxide, gepirone, and imipramine) supports this interpretation.

2.3. The Mouse Defense Test Battery

The Mouse Defense Test Battery was developed specifically to provide a parallel mouse test to the rat Fear/Defense Test Battery and Anxiety/Defense Test Battery. Use of the (rat-scaled, i.e. 6 m long) Fear/Defense Test Battery with mice (Blanchard et al., 1995a) found flight and escape responses when the mice were chased and freezing when the oval runway was converted to a straight alley. In contrast to rats, mice were invariably able to evade close contact with an oncoming experimenter so that defensive threat and attack were not seen in this situation. When confined and approached by an anesthetized rat, they did show defensive threat and attack. Thus, except for the inability of the experimenter to corner and contact the mouse subject in this large alleyway, these responses appeared to be virtually identical to those of rats. Two mouse strains were compared in this study, and Swiss–Websters tended to show more defenses than C57BL/6N mice as did females compared to males.

However, other studies provided important indications of differences in mouse and rat defensive behaviors. A mouse Visible Burrow System study (Blanchard et al., 1995b) indicated that mice, unlike rats, display risk assessment behaviors in the presence of the cat itself. When the cat is placed in the surface area of the Visible Burrow System, mice do flee to the tunnels but quickly return to the surface area entries to briefly peer out at the cat before turning and running into the depths of the tunnels again. Each mouse does this several times in 10 min or so after the cat is introduced, whereas rats never do so. This burst of risk assessment activity is short-lived and the mice soon regroup to huddle together for hours before returning to the surface, but it did suggest that risk assessment might be embedded in the actual response of mice to the predator.

And so it proved. The Mouse Defense Test Battery represented first a scaling-down of the Fear/Defense Test Battery to fit the much smaller dimensions of mice compared to rats. Second, for obvious reasons of scale, an anesthetized rat was used in place of the human experimenter as a threat stimulus. This can be justified on the basis that rats are predators of mice (Nikulina, 1991) and that mice show an unconditioned aversive/defensive reaction to them (De Catanzaro, 1988). Third, development of the Mouse Defense Test Battery involved additional examination of the behaviors of the mice while fleeing from the rat, and this yielded information on three behaviors that had not been observed during flight of rats from a chasing experimenter. While in flight, the mouse subjects would sometimes abruptly stop, look back at

the oncoming rat, and even reverse direction, attempting to run past it. The "stop" and "orientation" components of this behavior appeared to relate to risk assessment, i.e. gathering of information about the threat stimulus, while the third was less certain, either risk assessment or a new flight tactic. Similarly, while freezing in the straight alley to a more slowly approaching threat, mice sometimes moved toward the rat, then reversed course to run back to the end of the alley. This was measured as the number of such "approach–withdrawal" movements and as time out of the first square of the alley. The latter measure reflected that mice approached by a rat in a straight alley take up a position in the end ("first") square of the alley, which is divided into squares by lines on the floor. Time out of this square is a measure of approach to the rat.

It will have been noticed that the finding of risk assessment in mice during two separate phases of a Fear/Defense Test Battery-like protocol obviates the use of a separate set of Anxiety/Defense Test Battery-like procedures for eliciting these behaviors. The Mouse Defense Test Battery did not provide separate measures of behavioral inhibition, but following the logic outlined earlier, such behavioral inhibition appears to be strongly and perhaps nonspecifically associated with defensive behaviors, such that the individual evaluation of these makes its measurement less relevant. Similarly, the use of a much smaller runway/alley than that of the Fear/Defense Test Battery made it more difficult for mice to evade the oncoming threat source, with the result that they now showed reasonable levels of both defensive (sonic) threat vocalizations and actual biting attack to an oncoming rat. The behaviors evaluated in the Mouse Defense Test Battery thus constituted those taken directly from the rat Fear/Defense Test Battery (flight, measured as avoidance frequency and the distance between the threat stimulus and the subject when avoidance occurred); also, average and maximal flight speed (measured over a straight section of the alley), freezing, defensive threat (vocalizations) and defensive attack (jump attacks, biting), the risk assessment responses measured in the chase–flight (oval runway) and straight alley portions of the test, and a new "contextual defense" measure that involved attempts to escape the alley after the rat protocol had been completed. This could be compared to escape attempts during an initial period of identical length at the beginning of the test (before introduction of the rat) to assess whether rat confrontation had produced defensiveness to the apparatus (context) in which the rat had been presented. This pre-period also served as a measure of activity (line crossings) prior to rat exposure.

3. The Mouse Defense Test Battery: an experimental model of different emotional states: evidence from factor analysis

Factor analyses are commonly used to describe the relationship between different variables and, consequently, to identify specific indices or factors such as anxiety and locomotor activity. Thus, the question whether the different defensive responses elicited in the Mouse Defense Test Battery provide different measures of the same state or measure distinct states of defensiveness, fear, or anxiety has been approached by performing a factor analysis of the various behavioral defense reactions observed in the battery. The factor analysis identified four main independent factors (Griebel et al., 1996a). Factor 1 included cognitive aspects of defensive behaviors that appear to be related to the process of acquiring and analyzing information in the presence of threatening stimuli (i.e. risk assessment). Flight responses heavily loaded on Factor 2. Several defensive threat/attack reactions (i.e. upright postures and biting) highly loaded on Factor 3, indicating that this factor reflects more affective-orientated defense reactions. Finally, Factor 4, which includes escape attempts in the absence of the rat, relates to contextual defensiveness. Together, this pattern is consistent with the idea that defense reactions of mice exposed to a threat stimulus may relate to different emotional states and perhaps that they may model different aspects of human anxiety.

To address this hypothesis further, a variety of different clinically effective and marketed anxiolytic agents have been tested in the Mouse Defense Test Battery. As will be shown below, the pharmacological findings with the Mouse Defense Test Battery suggest that certain defensive behaviors may be considered particularly relevant in modelling specific aspects of anxiety disorders. For example, the observation that there is a rather good correspondence in terms of drug effects between the clinical outcome in panic disorder and generalized anxiety disorder and the ability to modify flight and risk assessment responses, respectively, suggests that the latter behaviors may be considered particularly relevant in modelling some aspects of panic disorder or generalized anxiety disorder. Moreover, previous reports have suggested that there may be an isomorphism between risk assessment in the Mouse Defense Test Battery and several behaviors often described in generalized anxiety disorder patients (Blanchard et al., 1997b) such as apprehensive expectation and vigilance and scanning involving hyper-attentiveness (DSM-IV, 1994). In addition, the observation that panic disorder patients usually report an urgent desire to flee from where the attack is occurring (DSM-IV, 1994), has led to suggestions that panic symptoms are due to pathological, spontaneous activation of neuronal mechanisms underlying flight reactions (Deakin and Graeff, 1991; Graeff, 1990). As such, flight behaviors in the Mouse Defense Test Battery may model certain aspects of panic (Griebel et al., 1996b).

4. $GABA_A$–benzodiazepine receptor ligands

Introduced over 40 years ago, benzodiazepines quickly became the most widely used of all psychotropic drugs.

Their marked anxiolytic, hypnotic, anticonvulsant, and muscle relaxant properties and their relative safety rapidly elevated benzodiazepines to the treatment of choice for common and recurrent conditions such as generalized anxiety disorder, muscle tension, and insomnia. However, these compounds have come under critical review because of the problems of drug dependence, tolerance, suppression of rapid eye movement sleep, rebound insomnia, and amnesia (Lader, 1994; Woods and Winger, 1995). Benzodiazepines produce their pharmacological effects by allosterically and positively modulating the action of GABA at $GABA_A$ receptors at specific ionotropic sites (Squires et al., 1979). Over the last two decades, a search has been undertaken for compounds chemically unrelated to benzodiazepines that may produce fewer unwanted effects but retain therapeutic properties. This search has led to the development of drugs that selectively bind to specific $GABA_A$ receptor subtypes (e.g. the hypnoselective agent zolpidem, which recognizes preferentially the α_1-containing $GABA_A$ receptor) (Depoortere et al., 1986) and/or show different efficacies at $GABA_A$ receptors (e.g. bretazenil, imidazenil, SL651498) (Giusti et al., 1993; Griebel et al., 2001b; Martin et al., 1988). The heterogeneity of $GABA_A$ receptors has prompted speculation that a particular behavioral response might be associated with an action at a defined receptor subtype. This idea has been substantiated by several findings using either subtype-selective $GABA_A$ receptor ligands or mutant mice in which a specific $GABA_A$ receptor subunit is inactivated. For example, the anxiolytic-like action of the nonselective $GABA_A$ receptor agonist diazepam is absent in α_2 but not in α_1 or α_3 knock-in mice, suggesting a major role for the α_2-containing $GABA_A$ receptor in the anxiolytic activity of benzodiazepines (Rudolph et al., 1999). This idea was also explored by using the Mouse Defense Test Battery with a variety of compounds that either bind nonselectively to $GABA_A$ receptor subtypes or show subtype-selective affinity and/or efficacy to a defined $GABA_A$ receptor subtype (Table 1).

4.1. Effects of nonselective $GABA_A$–benzodiazepine receptor ligands in the Mouse Defense Test Battery

Nonselective $GABA_A$–benzodiazepine receptor full agonists produce complex, but relatively similar, patterns of changes in defensive behaviors and, as it is discussed below, these changes differ somewhat from those seen with nonselective $GABA_A$–benzodiazepine receptor partial agonists and subtype-selective $GABA_A$–benzodiazepine receptor ligands. Results indicated that full agonists reduced markedly risk assessment activities observed in the chase test, defensive threat-attack reactions induced by physical contact with the rat, and escape attempts after the rat had been removed from the test area. Flight behaviors in response to the approaching rat were also reduced by these compounds, but to a lesser extent. Only the high-potency benzodiazepines, alprazolam, clonazepam, and triazolam, produced clear-cut and specific (i.e. at non-motor-impairing doses) reductions in this defensive response. Risk assessment, displayed when mice were constrained in a straight alley, was not modified by the full agonists, except by clobazam and diazepam. This difference in drug effects may be explained in part by the use of a modified version of the straight alley situation. In the earlier versions, the straight alley test was divided into three 15-s phases, using each a different distance between the mouse and the rat (i.e. 1.2, 0.8, and 0.4 m), whereas in the currently used version, the test consists of a single 30-s phase in which the rat stays at a constant distance of 0.4 m. It is possible that in the former situation, baseline levels of approaches followed by withdrawals (about three) were too high to be further increased, unlike the present version where baseline levels are close to zero. Together, these results indicate that defensive risk assessment, threat-attack reactions, and escape attempts show a consistent response to drugs used in the treatment of generalized anxiety disorder. Moreover, assuming that flight is an index of panic-like reactions, the findings of a lesser efficacy of nonselective $GABA_A$–benzodiazepine receptor full agonists against flight are in agreement with clinical data indicating that these drugs are generally of limited utility in the management of panic disorder (Pollack and Rosenbaum, 1988).

Results obtained with nonselective $GABA_A$–benzodiazepine receptor partial agonists in the Mouse Defense Test Battery indicate that these drugs displayed weaker efficacy in affecting defensive behaviors than full agonists. Except for a clear reduction in risk assessment displayed in the chase test, these compounds modified only weakly defensive aggression, contextual anxiety, and flight (Table 1). Interestingly, among the partial agonists tested in the Mouse Defense Test Battery, those displaying the lowest efficacy on the $GABA_A$–benzodiazepine receptor complex (e.g. flavonoids, bretazenil) were also the least effective. Obviously, a high intrinsic efficacy at the $GABA_A$–benzodiazepine receptor is required to affect the full-range of defensive behaviors elicited in the Mouse Defense Test Battery. Overall, the profile displayed by the nonselective $GABA_A$–benzodiazepine receptor partial agonists in the Mouse Defense Test Battery suggests a weak potential of these drugs in anxiety disorders. Although these compounds have been available for some time (Haefely et al., 1990) and a few clinical trials with such agents have been carried out, little is known of their efficacy in the treatment of anxiety disorders (Potokar and Nutt, 1994). It can be speculated that clinical results with these drugs were not disclosed because of their failure to show anxiolytic-like activity, thereby corroborating the findings from the Mouse Defense Test Battery.

Two other nonselective $GABA_A$–benzodiazepine receptor ligands have been tested in the Mouse Defense Test Battery, the antagonist, flumazenil, and the inverse agonist Ro 19-4603. Both compounds produced a mixed profile of increased and decreased defensiveness, depending on the

Table 1
Minimal effective doses (mg/kg, i.p., p.o., or s.c.) and efficacies of a variety of different benzodiazepine receptor ligands in the Mouse Defense Test Battery

	Flight	Risk assessment		Defensive aggression	Contextual defense	Locomotor activity	Reference
	Rat avoidance	Chase	Straight alley	Forced contact	Posttest	Pretest	
Alprazolam (acute), full agonist	1 (+)	1 (+)	>1	1 (+++)	1 (++)	1	Griebel et al. (1995c)
Alprazolam (repeated), full agonist	0.5 (+++)	0.5 (+)	>2	1 (+++)	2 (++)	>2	Griebel et al. (1995c)
Chlordiazepoxide, full agonist	2.5 (+)	10 (++)	>25	25 (+++)	25 (+++)	25	Griebel et al. (1996d)
Clobazam, full agonist	1 (++)	1 (+++)	10 (++)	1 (+++)	10 (+++)	10	Griebel et al. (1999a)
Clonazepam, full agonist	0.3 (+++)	0.1 (+++)	>1	0.3 (+++)	0.3 (+++)	>1	Griebel et al. (1996d)
Clorazepate, full agonist	0.3 (++)	3 (++)	>10	3 (+++)	3 (+++)	10	Griebel et al. (1996d)
Diazepam (acute), full agonist	3 (+)	1 (+++)	0.5 (++)	3 (+++)	1 (++)	>3	Griebel et al. (1998b)
Diazepam (repeated), full agonist	3 (+)	3 (+++)	>3	3 (+++)	>3	>3	Griebel et al. (2001b)
RWJ-46771, full agonist	0.1 (+++)	0.01 (+++)	>0.3	0.1 (++)	0.1 (+++)	0.03	Griebel et al. (1999a)
Triazolam, full agonist	0.03 (+++)	0.03 (+++)	>1	0.03 (+++)	0.03 (+++)	0.3	Griebel et al. (1998c)
Zopiclone, full agonist	>30	10 (+++)	>30	10 (+++)	30 (++)	10	Griebel et al. (1998c)
6-Br-flavone, partial agonist	>3	0.3 (+)	>3	>3	>3	>3	Griebel et al. (1999c)
6-Br-3-nitroflavone, partial agonist	>1	0.0001 (++)	>1	0.0001 (+)	>1	>1	Griebel et al. (1999c)
Bretazenil, partial agonist	1 (+)	1 (++)	>30	10 (+)	>30	>30	Griebel et al. (1996d)
Dinitroflavone, partial agonist	>1	>1	>1	0.01 (+)	>1	>1	[a]
Imidazenil, partial agonist	>10	0.3 (+++)	>10	10 (+)	1 (+)	>10	Griebel et al. (1996d)
Ro 19-8022, partial agonist	0.5 (++)	0.5 (+++)	>2	0.5 (+++)	>2	>2	Griebel et al. (1995b)
Y-23684, partial agonist	30 (+)	1 (+++)	10 (++)	10 (+++)	30 (+)	>30	Griebel et al. (1999a)
Flumazenil, antagonist	20 (− −)	>20	>20	5 (+)	10 (+)	>10	Griebel et al. (1995b)
Ro 19-4603, inverse agonist	0.025 (−)	0.025 (−)	>0.1	0.05 (−)	0.025 (++)	0.05	Griebel et al. (1995b)
Abecarnil, α_1 full agonist	0.1 (+)	0.3 (++)	>3	1 (+)	0.3 (+++)	0.3	Griebel et al. (1996d)
SX-3228, α_1 full agonist	0.03 (++)	0.3 (++)	>1	>1	1 (+++)	>1	Griebel et al. (1998c)
Zaleplon, α_1 full agonist	3 (+)	10 (+)	>10	3 (+++)	3 (+++)	>10	Griebel et al. (1998c)
Zolpidem, α_1 full agonist	3 (+)	>3	>3	3 (+)	>3	3	Griebel et al. (1996d)
CL218,872, α_1 partial agonist	>10	10 (+)	>10	>10	10 (+)	>10	Griebel et al. (1996d)
β-CCT, α_1 antagonist	>10	>10	>10	>10	>10	>10	[a]
SL651498 (acute), α_1 full agonist	10 (++)	10 (+++)	>10	10 (+++)	10 (+)	>10	Griebel et al. (2001b)
SL651498 (repeated), α_1 full agonist	10 (++)	3 (+++)	>10	3 (+++)	>10	>10	Griebel et al. (2001b)

+: Weak anxiolytic-like effects. ++: Significant anxiolytic-like effects. +++: Clear anxiolytic-like effects. −: Slight anxiogenic-like effects. − −: Significant anxiogenic-like effects.

[a] Unpublished data.

phase. The effects obtained with Ro 19-4603 on flight, risk assessment, and defensive aggression are consistent with an anxiogenic-like action of the drug since it increased further these behavioral measures. Anxiogenic-like effects of Ro 19-4603 have been observed in a previous study using the mouse light/dark choice paradigm (Belzung et al., 1990). The apparent paradoxical effect seen with Ro 19-4603 on contextual anxiety (i.e. decrease in escape attempts) has been suggested to reflect some relatively general reduction in locomotion (Griebel et al., 1995b). Flumazenil did not affect the risk assessment measures, but it dramatically potentiated flight reactions while weakly, albeit significantly, reducing defensive aggression and contextual anxiety. The effects of flumazenil on flight may well fit with clinical observations that the drug is somewhat anxiogenic in healthy volunteers (Darragh et al., 1983; Duka et al., 1986; Schopf et al., 1984) and that it increases the frequency of panic attacks in panic disorder patients (Maddock, 1998; Nutt et al., 1990; Woods et al., 1991). The weak agonist-like activity of flumazenil on defensive aggression is at first glance surprising, but it may fit with the idea that the drug can produce a $GABA_A$–benzodiazepine receptor set point shift either toward the agonistic or the inverse agonistic direction, depending on the aversiveness of the situation (Belzung et al., 2000).

4.2. Effects of subtype-selective $GABA_A$–benzodiazepine receptor ligands in the Mouse Defense Test Battery

A wide variety of ligands are now known to interact selectively with $GABA_A$–benzodiazepine receptor subtypes, and the field is being researched with increased vigour in an effort to produce more selective agents. While there are $GABA_A$–benzodiazepine receptor ligands claimed in patents or shown to bind selectively for all benzodiazepine-sensitive $GABA_A$ receptor subtypes, mostly compounds selective for the α_1 receptor subtype have been studied in vivo (Griebel et al., 2000a). Several of these drugs have been investigated in the Mouse Defense Test Battery (Table 1). At non-motor impairing doses, the α_1 receptor subtype compounds either failed to affect flight (e.g. zolpidem, β-CCT) or weakly (e.g. abecarnil, CL 218,872, and zaleplon) reduced some but not all flight measures. These results on flight indicate that the α_1 receptor subtype cannot be considered as the primary target mediating the flight-reducing action of drugs interacting with the $GABA_A$–benzodiazepine receptor complex and subsequently suggest that panic responses may not involve the α_1 receptor subtype. Only abecarnil and SX-3228 clearly decreased risk assessment activities during the chase test but the former only at motor-impairing doses as revealed by the pretest. Other compounds such as CL 218,872 and zaleplon weakly affected risk assessment during the chase test, while zolpidem, the most selective α_1 receptor subtype agonist tested in the Mouse Defense Test Battery so far, failed to alter risk assessment measures, suggesting that the α_1 receptor subtype does not mediate an effect on this particular defense response. Results from the forced contact and posttests are consonant with these findings as the α_1 receptor subtype ligands either reduced some defensive reactions in a nonspecific manner (e.g. zolpidem and abecarnil) or were devoid of any effects on these responses (e.g. CL 218,872, β-CCT). The only exception was zaleplon, which decreased both defensive reactions at doses much lower than those impairing general activity. Overall, however, the findings with α_1-subtype ligands in the Mouse Defense Test Battery demonstrated neither clear effects nor specific action on defensive reactions, suggesting that the defense system does not primarily involve $GABA_A$–benzodiazepine receptors bearing the α_1 unit.

Based on recent findings with mice having point-mutated diazepam-insensitive $GABA_A$–benzodiazepine receptor subtypes, which showed that the anxiolytic-like effects of $GABA_A$–benzodiazepine receptor agonists are mediated by the α_2 $GABA_A$–benzodiazepine receptor (Low et al., 2000; Rudolph et al., 1999), research for anxioselective compounds acting at the $GABA_A$–benzodiazepine receptor subtypes has focused on the development of ligands that display functionally selective agonist activity at the α_2 $GABA_A$ receptor subtype. The recently discovered pyridoindole derivative SL651498 fulfills this criterion. In animal experiments, SL651498 elicited anxiolytic-like activity qualitatively and quantitatively similar to that of benzodiazepines, but unlike these latter, induced central depressant effects at doses much higher than those producing anxiolytic-like activity (Griebel et al., 2001b). When tested in the Mouse Defense Test Battery, SL651498 markedly modified defensive behaviors (Table 1). Prominent effects of the drug were observed on risk assessment activities and defensive attack reactions, but unlike the nonselective $GABA_A$–benzodiazepine receptor full agonists, SL651498 did not modify contextual anxiety. Despite this latter observation, the overall profile of SL651498 in the Mouse Defense Test Battery points to a major role for the $GABA_A$ α_2 subtype in regulating defensive behaviors.

5. Selective and nonselective 5-HT-interacting drugs

Although benzodiazepines remain the mainstay of the treatment of anxiety disorders, preclinical research in this area has mainly focused on compounds modulating 5-HT (5-hydroxytryptamine) neurotransmission during the last two decades (Griebel, 1995; Griebel, 1997). However, it is somewhat surprising to note that after all this research effort, only a few direct 5-HT-acting compounds have been launched for the treatment of generalized anxiety disorder (i.e. buspirone and tandospirone) (Barradell and Fitton, 1996; Fulton and Brogden, 1997). In addition, only selective 5-HT re-uptake and monoamine-oxidase inhibitors have been successfully used in the chronic treatment of panic attacks, obsessive–compulsive, and posttraumatic stress

disorders (Billett et al., 1997; Buller, 1995; Fichtner et al., 1997; Liebowitz et al., 1990; Priest et al., 1995; Westenberg, 1996). Although interest in this research area has steadily decreased, novel 5-HT-modulating agents are still being developed. Much attention has focused on the behavioral effects of selective 5-HT_{1A} receptor ligands, but interest in drugs combining 5-HT_{1A}, 5-HT_2, and/or 5-HT re-uptake inhibitor properties is increasing. The effects on defensive behaviors of a variety of these drugs have been investigated in the Mouse Defense Test Battery (Table 2).

5.1. Effects of selective 5-HT_{1A} receptor ligands in the Mouse Defense Test Battery

The administration of 5-HT_{1A} receptor agonists, regardless of their intrinsic efficacy (i.e. partial or full), had no significant or specific effect on flight and risk assessment (Table 1). While flight and risk assessment during the chase test remained unaffected up to very high doses, risk assessment elicited in the straight alley situation was decreased by 8-OH-DPAT and gepirone at doses that also dramatically reduced locomotor activity, thereby suggesting a nonspecific action on this defensive behavior. This was in contrast to the effects observed in the forced contact and posttests, where all 5-HT_{1A} receptor agonists tested clearly and specifically reduced defensive aggression and escape attempts, respectively. Although 5-HT_{1A} receptor agonists have no myorelaxant or ataxic effects, the severe reduction in activity seen in the pretest may be indicative of the appearance of the 5-HT syndrome, described in detail in subsequent studies using the runway apparatus (Blanchard et al., 1997a). Based on the idea that flight may model certain aspect of a panic attack, the failure of 5-HT_{1A} receptor agonists to modify this defensive reaction is not unexpected since human studies provide undisputed evidence of a lack of efficacy of such agents in panic reactions (Pecknold et al., 1993; Pohl et al., 1989; Sheehan et al., 1990; Van Vliet et al., 1996; Westenberg et al., 1992). Despite their clear effects on defensive aggression, a behavior proposed to reflect affective oriented aspects of generalized anxiety disorder, it is intriguing and puzzling that 5-HT_{1A} receptor agonists did not affect the highly benzodiazepine-sensitive risk assessment measure during the chase test. Although 5-HT_{1A} receptor agonists have been found effective against generalized anxiety disorder (Apter and Allen, 1999; Cutler et al., 1993; Rickels et al., 1997), it is possible that there may be important differences between anxiety that is responsive to benzodiazepines as opposed to anxiety responsive to 5-HT_{1A} receptor agonists. However, this issue has been relatively little investigated on the human level. Unlike benzodiazepines, 5-HT_{1A} anxiolytics are active in the clinic only after repeated administration. It can, therefore, be speculated that 5-HT_{1A} receptor agonists would have produced a broader spectrum of activity on defensive behaviors in the Mouse Defense Test Battery after chronic administration. Unfortunately, no such experiments have been performed so far to verify this idea.

Studies using traditional rodent models of anxiety, such as the elevated plus-maze or conflict procedures, have demonstrated that the anxiety-reducing potential of 5-HT_{1A} receptor antagonists may be superior to that of full or partial agonists for this receptor (Cao and Rodgers, 1997a–c; Griebel, 1999a; Griebel et al., 2000b). Results obtained in the Mouse Defense Test Battery with 5-HT_{1A} receptor antagonists largely agree with these findings (Table 2). Overall, the profiles displayed by selective (e.g. WAY 100635, *p*-MPPI, and SL88.0338) and, to a lesser extent, nonselective 5-HT_{1A} receptor (e.g. NAN-190, MM-77) antagonists in the Mouse Defense Test Battery are comparable to that of benzodiazepines, although the magnitude of the effects of the 5-HT_{1A} compounds was generally smaller. With the exception of one risk assessment measure (i.e. approach/withdrawal behavior in the straight alley test), selective 5-HT_{1A} receptor antagonists affected all other defensive responses in the presence of the threat stimulus. However, unlike 5-HT_{1A} receptor agonists and benzodiazepines, these compounds attenuated contextual anxiety only at high and mostly motor-impairing doses. Despite the latter result, these findings demonstrate that selective 5-HT_{1A} receptor antagonists produced clear anxiolytic-like effects in the Mouse Defense Test Battery. The precise mechanisms underlying the positive effects of 5-HT_{1A} receptor antagonists in anxiety models remain to be determined. These compounds have all demonstrated antagonistic-like activity on pre- and postsynaptic 5-HT_{1A} receptors. Based on the findings that exposure to aversive stimuli as in anxiety models increases 5-HT release, we would expect a 5-HT_{1A} receptor antagonist to attenuate this effect and, thus, display anxiolytic activity. However, further studies on this issue are clearly warranted. Clinical trials with 5-HT_{1A} receptor antagonists in patients with anxiety disorders will eventually determine whether such compounds may be useful in the treatment of these conditions.

5.2. Effects of selective and nonselective 5-HT_2 receptor antagonists in the Mouse Defense Test Battery

Evidence supporting a role for 5-HT_2 receptors in anxiety mainly arises from early clinical observations showing that the nonselective 5-$HT_{2A/2B/2C}$ receptor antagonist ritanserin was shown to be effective in improving several anxiety disorders, including panic and generalized anxiety disorder (Ceulemans et al., 1985; Humble et al., 1986). However, negative results with ritanserin and other 5-HT_2 receptor antagonists have been reported in subsequent clinical trials (Den Boer and Westenberg, 1990; Sramek et al., 1995; Westenberg and Den Boer, 1989). In behavioral tests with laboratory animals, the effects of 5-HT_2 receptor antagonists on anxiety-related behaviors are highly variable (Griebel, 1995, 1997). The few 5-HT_2 receptor antagonists tested in the Mouse Defense Test Battery did not reveal reliable effects of

Table 2
Minimal effective doses (mg/kg, i.p., p.o., or s.c.) and efficacies of a variety of different selective and nonselective 5-HT-interacting drugs in the Mouse Defense Test Battery

	Flight	Risk assessment		Defensive aggression	Contextual defense	Locomotor activity	Reference
	Rat avoidance	Chase	Straight alley	Forced contact	Posttest	Pretest	
8-OH-DPAT, 5-HT_{1A} full agonist	>10	>10	0.5 (?)	0.5 (+++)	0.5 (+++)	0.5	Griebel et al. (1995d)
Buspirone, 5-HT_{1A} partial agonist	>5	>5	>5	1.25 (+++)	1.25 (+++)	5	Griebel et al. (1998b)
Gepirone, 5-HT_{1A} partial agonist	>10	>10	5 (?)	2.5 (+++)	0.5 (+++)	5	Griebel et al. (1995d)
MM-77, 5-HT_{1A} antagonist	1 (++)	0.3 (++)	>1	0.1 (++)	0.1 (+++)	0.1	Griebel et al. (1999d)
NAN-190, 5-HT_{1A} antagonist	1 (++)	0.1 (++)	>3	0.3 (+++)	3 (+)	>3	Griebel et al. (1999d)
p-MPPI, 5-HT_{1A} antagonist	1 (+)	0.3 (++)	>3	3 (+)	3 (+)	3	Griebel et al. (1999d)
Pindobind-5-HT_{1A}, 5-HT_{1A} antagonist	>1	0.03 (++)	0.1 (+)	1 (+)	>1	>1	Griebel et al. (1999d)
S21187, 5-HT_{1A} antagonist	10 (+)	>10	>10	2.5 (+)	2.5 (+++)	>10	[a]
SL88.0338, 5-HT_{1A} antagonist	1 (++)	0.3 (++)	>3	1 (++)	>3	>3	Griebel et al. (1999d)
(*S*)-UH-301, 5-HT_{1A} antagonist	2 (++)	0.3 (++)	>3	2 (+)	1 (++)	3	Griebel et al. (1999d)
WAY100635, 5-HT_{1A} antagonist	1 (++)	0.01 (++)	>3	0.1 (+++)	3 (+)	3	Griebel et al. (1999d)
Mianserin, 5-HT_{2ABC} antagonist	>10	>10	10 (?)	>10	>10	3	Griebel et al. (1997a)
SB 206553, 5-HT_{2BC} antagonist	>20	>20	20 (?)	20 (+)	20	20	Griebel et al. (1997a)
MDL 100,907, 5-HT_{2A} antagonist	>3	1 (+)	>3	>3	>3	>3	Griebel et al. (1997a)
Pirenperone, 5-HT_{2A} antagonist	0.25 (+++)	>10	1 (?)	1 (++)	0.5 (++)	0.5	Griebel et al. (1995d)
S21357, 5-$HT_{1A/2A}$ antagonist	0.125 (++)	0.125 (+)	>2	0.125 (++)	0.125 (++)	>2	Griebel et al. (1996c)
Fluoxetine (acute), 5-HT re-uptake inhibitor	5 (−)	>15	>15	15 (− −)	10 (+)	>15	Griebel et al. (1995a)
Fluoxetine (repeated), 5-HT re-uptake inhibitor	5 (+++)	5 (++)	>15	5 (++)	5 (++)	>15	Griebel et al. (1995a)
Imipramine (acute), 5-HT/NA uptake inhibitor	5 (− −)	>15	>15	5 (−)	>15	>15	Griebel et al. (1995a)
Imipramine (repeated), 5-HT/NA uptake inhibitor	5 (+++)	10 (++)	>15	5 (+++)	5 (++)	>15	Griebel et al. (1995a)
Befloxatone (acute), MAO_A inhibitor	>1	>1	>1	>1	>1	>1	Griebel et al. (1997b)
Befloxatone (repeated), MAO_A inhibitor	1 (+)	>1	0.3 (++)	>1	>1	>1	Griebel et al. (1997b)
Moclobemide (acute), MAO_A inhibitor	>10	>10	>10	>10	>10	>10	Griebel et al. (1997b)
Moclobemide (repeated), MAO_A inhibitor	3 (+)	>10	>10	>10	>10	>10	Griebel et al. (1997b)
Phenelzine (acute), MAO_{AB} inhibitor	30 (+)	>30	>30	>30	30 (+)	30	Griebel et al. (1998a)
Phenelzine (repeated), MAO_{AB} inhibitor	10 (++)	>30	10 (++)	>30	30 (++)	30	Griebel et al. (1998a)

+: Weak anxiolytic-like effects. ++: Significant anxiolytic-like effects. +++: Clear anxiolytic-like effects. − : Slight anxiogenic-like effects. − − : Significant anxiogenic-like effects. ?: Effect probably not related to the modulation of anxiety processes.
[a] Unpublished data.

these compounds on defensive behaviors (Table 2). Only the 5-HT_{2A} receptor antagonist pirenperone was able to attenuate several defensive reactions, including flight, defensive aggression, and contextual anxiety. While the effect on flight was observed at non-motor-impairing doses, the action of pirenperone on the two latter defensive responses occurred at

doses that did also significantly reduce pretest locomotor activity, indicating a nonspecific action of the drug. The effects of pirenperone on flight were not confirmed with another 5-HT_{2A} receptor antagonist MDL 100,907, which was devoid of activity on flight as well as on all other Mouse Defense Test Battery measures. Furthermore, in view of the profiles displayed by selective 5-HT_{1A} receptor antagonists in the Mouse Defense Test Battery (see above), the marked and specific attenuation of most defensive behaviors seen following the administration of the mixed 5-$HT_{1A/2A}$ receptor antagonist S21357 is probably due to its antagonistic action at the 5-HT_{1A} receptor.

5.3. Effects of 5-HT re-uptake and monoamine-oxidase inhibitors in the Mouse Defense Test Battery

Because clinical data indicate that selective 5-HT re-uptake inhibitors and monoamine-oxidase inhibitors require long-term treatment to achieve therapeutic response, these compounds were administered both acutely and repeatedly in the Mouse Defense Test Battery. After single administrations, the mixed 5-HT/NA re-uptake inhibitor imipramine, the selective 5-HT re-uptake inhibitor fluoxetine, and the monoamine oxidase inhibitors befloxatone, moclobemide, and phenelzine did not decrease any of the defense responses in a specific manner (Table 2). Instead, imipramine and fluoxetine potentiated flight responses and defensive biting. In sharp contrast with this profile, chronic administration of the two drugs decreased both measures. In addition, imipramine and fluoxetine also decreased risk assessment activities when subjects were chased by the rat and escape attempts after the removal of the rat. After repeated administration of the monoamine-oxidase inhibitors, a significant reduction in flight was observed. In addition, befloxatone and phenelzine increased risk assessment responses when mice were constrained in one part of the apparatus facing the rat, which remained at a constant distance. No other drug effects were observed with these compounds.

The efficacy of fluoxetine, imipramine, and phenelzine in the treatment of panic disorder is well established (Ashok Raj and Sheehan, 1995; Garakani et al., 1984; Solyom et al., 1991). As such, their clear effects on flight in the Mouse Defense Test Battery indicates further that this behavior may represent a relatively reliable measure of certain aspects of panic disorder. Interestingly, the behavioral changes produced by chronic phenelzine in the Mouse Defense Test Battery were associated with a dramatic increase in 5-HT levels, while levels of dopamine and norepinephrine increased only slightly (Griebel et al., 1998a). This result suggests that the effects of phenelzine on flight may be due mainly to its action on the 5-HT system, which is in agreement with the well-accepted idea that 5-HT plays a crucial role in panic disorder (Bell and Nutt, 1998). Furthermore, some of these compounds (i.e. imipramine, fluoxetine, and phenelzine) partially affected defensive threat/attack responses and/or risk assessment activities, effects that may also corroborate clinical findings showing that these drugs have a broad spectrum of therapeutic activity against anxiety disorders (Modigh, 1987; Zohar and Westenberg, 2000). In addition, the finding of a potentiation in some defense reactions (i.e. flight and bitings) after a single dose of imipramine and fluoxetine fits well with the clinical observation of an exacerbation in anxious responses, which may sometimes occur at the beginning of treatment with imipramine or with a selective 5-HT re-uptake inhibitor (Westenberg, 1996; Westenberg and Den Boer, 1993).

6. Neuropeptides and neuropeptide receptor ligands

The treatment of anxiety disorders remains an active area of research, and anxiolytic drug discovery focuses more and more on the involvement of neuroactive peptides in the modulation of anxiety behaviors. Among these, corticotropin-releasing factor (CRF), cholecystokinin (CCK), and tachykinins (substance P and neurokinin A and B) have been the most extensively studied, but the involvement of other neuroactive peptides such as neuropeptide Y, arginine vasopressin, nociceptin/orphanin FQ, and neurotensin has also been examined (Aguilera and Rabadan-Diehl, 2000; Griebel, 1999b; Rowe et al., 1995; Smith and Moran, 2001). Specific and highly potent non-peptide receptor ligands have been discovered and developed for most of these peptides (Betancur et al., 1997; Calo et al., 2000a; Dunlop, 1998; Gully et al., 1993; McCarthy et al., 1999; Serradeil-Le Gal et al., 2002). A few of them have been tested in the Mouse Defense Test Battery and as will be shown below, they yielded behavioral profiles in this procedure that differed from those observed with benzodiazepines.

6.1. Effects of CRF receptor antagonists in the Mouse Defense Test Battery

A rapidly increasing number of preclinical and clinical studies have emphasized the pivotal role of CRF in coordinating the overall response of the body to stressors (for a recent review, see Holsboer, 1999). Much of the evidence comes from experiments showing that intracerebroventricular application of CRF in rodents produces behavioral effects similar to those observed when animals are exposed to stress. Moreover, it is well acknowledged that CRF is the major hypophysiotropic factor regulating basal and stress-induced release of adrenocorticotropic hormone (ACTH) and β-endorphin (Vale et al., 1981, 1983). The effects of CRF are mediated by two specific G protein-coupled 7-transmembrane domain receptors called CRF_1 and CRF_2 (Chalmers et al., 1996). Tissue distribution analysis showed that CRF_1 receptor expression is most abundant in neocortical, cerebellar, and limbic structures, whereas CRF_2 receptor expression is generally localized in subcortical structures, notably in the lateral septum and various hypothalamic areas (Chalmers et al., 1995). This anatomical information provided a basis for

functional hypotheses related to CRF receptor subtypes and suggested that CRF may contribute significantly both to behavioral responses to stress and emotional behavior itself. As a result, it was hypothesized that CRF receptor antagonists may represent a novel class of agents for the treatment of anxiety disorders (Gutman et al., 2000).

Several synthetic CRF_1 receptor antagonists have been tested in the Mouse Defense Test Battery. Results showed that they attenuated some, but not all, defensive behaviors (Table 3). They produced inconsistent effects on flight and weakly affected risk assessment behaviors. While SSR12 5543A and CP-154,526 significantly reduced avoidance responses, antalarmin failed to modify these measures, and PD171,729 produced nonspecific effects. Furthermore, risk assessment displayed by mice in the chase test was significantly, albeit only weakly and non-dose-dependently, reduced by antalarmin and CP-154,526. When mice were constrained in a straight inescapable alley, the administration of the CRF_1 receptor antagonists did not produce significant changes in risk assessment activity. In contrast, upon forced contact with the rat, they all showed markedly reduced defensive aggression. These effects are unrelated to motor impairment, as activity measures recorded before the confrontation with the rat were not significantly altered by the drugs. Finally, after the removal of the rat from the apparatus, only PD171,729 and CP-154,526 significantly, but again non-dose-dependently, counteracted the dramatic increase in escape attempts. Overall, the profile displayed by CRF_1 receptor antagonists in the Mouse Defense Test Battery differed from that of benzodiazepines. While these

Table 3
Minimal effective doses (mg/kg, i.p., p.o., s.c., or i.c.v.) and efficacies of a variety of different neuropeptides and neuropeptide receptor ligands in the Mouse Defense Test Battery

	Flight	Risk assessment		Defensive aggression	Contextual defense	Locomotor activity	Reference
	Rat avoidance	Chase	Straight alley	Forced contact	Posttest	Pretest	
Antalarmin, CRF_1 antagonist	>30	10 (+)	>30	1 (+++)	>30	>30	Griebel et al. (2002b)
CP-154,526, CRF_1 antagonist	5 (++)	5 (+)	>20	10 (+++)	5 (+)	>20	Griebel et al. (1998b)
PD171,729, CRF_1 antagonist	10 (++)	10 (+)	>10	10 (++)	10 (++)	10	[a]
SSR125543A, CRF_1 antagonist	10 (++)	>30	>30	10 (+++)	>30	>30	Griebel et al. (2002b)
Lorglumide, CCK_1 antagonist	>10	>10	>10	>10	>10	>10	Griebel et al. (1997c)
LY 288513, CCK_2 antagonist	1 (++)	>3	>3	>3	>3	>3	Griebel et al. (1997c)
PD 135,158, CCK_2 antagonist	0.001 (++)	>1	>1	>1	>1	>1	Griebel et al. (1997c)
Substance P, NK_1 agonist	0.5 (+)	>1	>1	1 (++)	>1	>1	[a]
CP-96,345, NK_1 antagonist	1 (+)	1 (+)	>1	0.3 (++)	>1	>1	[a]
CP-99,994, NK_1 antagonist	1 (+)	1 (+)	>1	0.3 (++)	>1	>1	[a]
GR159897, NK_2 antagonist	0.01 (+)	0.01 (++)	1 (+)	0.1 (+++)	3 (+)	>3	[a]
SR48968, NK_2 antagonist	1 (+)	0.3 (+)	>1	0.03 (+++)	0.03 (+)	>1	Griebel et al. (2001c)
SR144190, NK_2 antagonist	3 (+)	>10	>10	3 (+++)	>10	>10	Griebel et al. (2001a)
SR48692, NT antagonist	10 (++)	>30	30 (+)	10 (+++)	1 (++)	10	Griebel et al. (2001a)
SSR149415, V_{1b} antagonist	30 (+)	>30	>30	1 (+++)	>30	>30	Griebel et al. (2002a)
Orphanin FQ/ nociceptin, OP_4 agonist	3 nM (++)	>3 nM	>3 nM	0.3 nM (+++)	>3 nM	3 nM	Griebel et al. (1999b)
Prepro-$TRH_{178-199}$, CRF release inhibitor	>12 μg	>12 μg	>12 μg	>12 μg	>12 μg	>12 μg	[a]
Tuftsin, peptide immunomodulator	10 (++)	3 (++)	30 (++)	3 (+++)	10 (++)	>30	[a]

+: Weak anxiolytic-like activity. ++: Significant anxiolytic-like effects. +++: Clear anxiolytic-like effects.
[a] Unpublished data.

latter drugs modified both cognitive and affective aspects of defensive behaviors and produced clear effects on contextual anxiety, and to a lesser extent, on flight, the CRF_1 receptor antagonists are mostly active on affective aspects of defensive behaviors (i.e. on terminal defense, when there is no possibility to escape, and confrontation with the threat stimulus becomes unavoidable).

6.2. Effects of CCK receptor antagonists in the Mouse Defense Test Battery

CCK is recognized as the most widely distributed neuropeptide in the brain (Hokfelt et al., 1985). Two forms of CCK receptors have been characterized pharmacologically for their responsivity to the sulfated (CCK_1) or unsulfated (CCK_2) forms of CCK (Moran et al., 1986). While CCK_1 receptors are expressed in the alimentary tract and discrete regions of the brain (e.g. area postrema, posterior hypothalamus, and nucleus accumbens), CCK_2 receptors are widely distributed in the central nervous system (e.g. limbic structures) (De Weerth et al., 1993; Pisegna et al., 1992). The neuroanatomical distribution of CCK has prompted speculation about its functional role in anxiety disorders and has fueled both basic research and commercial interest in the CCK system, leading to numerous studies that investigated the behavioral action of CCK fragments and CCK receptor ligands in animal models of anxiety (Griebel, 1999b).

In the Mouse Defense Test Battery, pretreatment with the CCK_1 receptor antagonist lorglumide did not modify any of the behavioral measures during the exposure to the rat (Table 3). After the administration of the CCK_2 receptor antagonists, PD 135,158 and LY 288513, flight responses were significantly decreased, while all risk assessment measures and defensive aggression remained unchanged. In addition, none of the CCK receptor antagonists were able to counteract the increase in escape attempts from the runway following the removal of the rat from the test area. The profiles displayed by the CCK receptor antagonists in the Mouse Defense Test Battery largely confirm findings from traditional anxiety tests, which indicated that these compounds have a weak anxiety-reducing potential (Griebel, 1999b). The positive effects of PD 135,158 and LY 288513 on flight, however, fit well with the general assumption that antagonists targeting CCK_2 receptors may have some efficacy against panic disorder. Although few studies demonstrated that the CCK_2 receptor antagonist L-365,260 reversed panic attacks elicited by pharmacological challenge (e.g. sodium lactate, CCK-4, and pentagastrin) (Bradwejn et al., 1994, 1995; Lines et al., 1995), two placebo-controlled trial of CCK_2 receptor antagonists on naturally occurring panic attacks failed to detect clinically significant differences between drug and placebo (Kramer et al., 1995; Pande, 1997). Therefore, it is not clear whether the effects of PD 135,158 and LY 288513 on flight are predictive of an antipanic-like action of the drugs or relate to behavioral processes not associated with anxiety.

6.3. Effects of tachykinin receptor ligands in the Mouse Defense Test Battery

The tachykinin neuropeptide family includes substance P, neurokinin A, and neurokinin B. Their biological actions are mediated via the activation of three receptors: the tachykinin NK_1, tachykinin NK_2, and tachykinin NK_3 receptors (Regoli et al., 1994). While tachykinin NK_1 and tachykinin NK_3 receptors are widely distributed in the CNS, the tachykinin NK_2 receptor is found centrally with considerably lower levels (Maggi, 1995; Otsuka and Yoshioka, 1993). All three types of tachykinin receptors are located in brain areas implicated in the control of fear and anxiety, such as the hypothalamus, amygdala, hippocampus, and periaqueductal gray matter (Otsuka and Yoshioka, 1993). The neuroanatomical distribution of tachykinin receptors has led to numerous studies that investigated the behavioral action of tachykinin receptor ligands in animal models of anxiety (Griebel, 1999b). An overview of these findings shows that unlike selective tachykinin NK_1 and tachykinin NK_3 receptor antagonists, which have variable effects in anxiety models, selective tachykinin NK_2 receptor antagonists produce clear anxiolytic-like activity, especially in models based on exploratory responses.

The administration of substance P, the preferred endogenous peptide for the NK_1 receptor, did not dramatically modify the behavior of mice in the Mouse Defense Test Battery, indicating that the modulation of defensive behaviors does not primarily involve the NK_1 receptor (Table 3). However, the drug produced a weak reduction in flight, and decreased defensive aggression at the highest dose was tested (1 mg/kg, s.c.). Surprisingly, the tachykinin NK_1 receptor antagonists, CP-96,345 and CP-99,994, produced similar reductions in flight and defensive aggression. A number of research groups have found that substance P displays anxiolytic-like effects, whereas others have reported a lack of activity or even an anxiogenic-like profile (Griebel, 1999b). It has been suggested that the effects of substance P in anxiety models may be dependent on dose, administration route, and specific brain region.

When tested in the Mouse Defense Test Battery, the selective tachykinin NK_2 receptor antagonists, GR159897, SR48968, and SR144190, decreased weakly flight, and the two former reduced risk assessment activities as well as contextual anxiety. When contact was forced between threat stimulus and subject, all three tachykinin NK_2 receptor antagonists markedly reduced bites to the rat. Overall, the behavioral profile displayed by the tachykinin NK_2 receptor antagonists in the Mouse Defense Test Battery is consistent with an anxiolytic-like action. However, while they are much less efficient than classical anxiolytics such as benzodiazepines on responses which include flight measures and cognitive aspects of defensive behaviors, they

appear to be as effective as benzodiazepines on more affective-orientated defense reactions. The exact mechanisms underlying the marked effects of tachykinin NK_2 receptor antagonists on defensive threat and attack behaviors remain to be determined. In the case of SR48968, the lack of significant effects in the Mouse Defense Test Battery of its (*R*)-enantiomer SR48965 (Griebel et al., 2001c), which shows only weak affinity for the tachykinin NK_2 receptor site, indicates that tachykinin NK_2 receptor blockade may be necessary to produce such effects. Moreover, it has been suggested that tachykinin NK_2 receptor antagonists may produce some of their in vivo effects by interacting with other neurotransmitters such as CRF (Steinberg et al., 2001). This idea may be substantiated by the observation that CRF_1 and tachykinin NK_2 receptor antagonists display quite similar profiles in the Mouse Defense Test Battery.

6.4. Effect of the neurotensin receptor antagonist SR48692 in the Mouse Defense Test Battery

The 13-amino-acid peptide neurotensin displays a wide spectrum of physiological activities, including notably a role as neuromodulator in the brain (McCann et al., 1982; Nemeroff et al., 1983; St. Pierre et al., 1980). The abundance of the peptide and its two receptors (i.e. neurotensin NT_1 receptor and neurotensin NT_2 receptor) in the hypothalamus and the central nucleus of the amygdala suggests a central role in the regulation of endocrine responses to external events or in the alteration of emotional tone, functions thought to be controlled by the amygdala (Roberts et al., 1982; Elde et al., 1990; Sarret et al., 1998; Walker et al., 1998). Central administration of neurotensin was reported to increase plasma levels of ACTH and corticosterone (Gudelsky et al., 1989; Nicot et al., 1994; Nicot et al., 1997). Psychological stressors, such as non-escapable tail electric shock in rats, were found to increase neurotensin mRNA within the medial parvocellular region of the paraventricular nucleus of the hypothalamus (Helmreich et al., 1999). Neurotensin receptor blockade by the non-peptide neurotensin NT_1 receptor antagonist SR48692 was found to attenuate restraint stress-induced elevations in hypothalamic pituitary adrenal activity (Rowe et al., 1997), but it was without effect in a variety of different classical animal models of anxiety in rodents (Griebel et al., 2001a). When tested in the Mouse Defense Test Battery, SR48692 decreased flight reactions, although the magnitude of the effects was less than that observed with benzodiazepines and antipanic agents (Table 3). During both the chase and the straight alley tests, SR48692 modified risk assessment activities. As was the case in the rat avoidance test, the magnitude of the effects of SR48692 was generally smaller than that of benzodiazepines. However, when contact was forced between threat stimulus and subject, SR48692 clearly reduced defensive threat and attack reactions. Finally, in the posttest, following removal of the rat from the apparatus, SR48692 decreased escape attempts from the test apparatus. Together, the data from the Mouse Defense Test Battery indicate that SR48692 has weak to no effects on several behavioral measures primarily modified by benzodiazepines. However, they indicate that neurotensin receptor blockade may play a role in the adaptive responses to unavoidable or extreme stress events. Previous studies have revealed the existence of an interplay between neurotensin and CRF at the level of the paraventricular nucleus of the hypothalamus (Nicot et al., 1997), leading to the possibility that SR48692 may exert its effects on defense via blockade of the increase in hypothalamic pituitary adrenal activity produced by rat exposure stress.

6.5. Effect of the arginine vasopressin V_{1b} receptor antagonist SSR149415 in the Mouse Defense Test Battery

The nonapeptide arginine vasopressin participates in the hypothalamic pituitary adrenal axis, regulating pituitary ACTH secretion by potentiating the stimulatory effects of CRF. Extra hypothalamic arginine vasopressin-containing neurons have also been characterized in the medial amygdala, which innervate limbic structures such as the lateral septum and the ventral hippocampus (De Vries and Buijs, 1983). In these latter regions, arginine vasopressin exerts a neuromodulatory role via an action on specific receptors, i.e. V_{1a} and V_{1b} (Lolait et al., 1995; Vaccari et al., 1998; Young et al., 1999), which are widely distributed in the central nervous system (Lolait et al., 1995; Morel et al., 1992). Several findings suggest that arginine vasopressin may be involved in the modulation of emotional processes primarily via the V_{1b} receptor subtype. For example, chronic immobilization stress has been shown to increase V_{1b} receptor mRNA levels in the rat brain (Aguilera and Rabadan-Diehl, 2000). Selective blockade of the V_{1b} receptor by the non-peptide antagonist SSR149415 was shown to block restraint stress-induced ACTH release (Serradeil-Le Gal et al., 2002) and produce anxiolytic-like activity in several procedures (Griebel et al., 2002a). The magnitude of these effects was overall less than that of the benzodiazepine anxiolytic diazepam in classical anxiety tests such as the elevated plus-maze. However, SSR149415 produced marked effects in models involving traumatic social confrontation. The results from the Mouse Defense Test Battery are in line with these findings as SSR149415 failed to modify significantly risk assessment and contextual anxiety and only weakly affected flight, but it produced clear-cut effects on defensive aggression when mice are directly confronted with the rat (Table 3).

6.6. Effects of the OP_4 endogenous peptide nociceptin/orphanin FQ in the Mouse Defense Test Battery

The heptadecapeptide nociceptin/orphanin FQ is a recently discovered neuropeptide that exhibits structural homology with the opioid peptides and binds to an opioid-like G protein-coupled receptor (called OP_4) (Calo

et al., 2000b; Meunier et al., 1995; Reinscheid et al., 1995). The neuroanatomical distribution of nociceptin/orphanin FQ and its receptor in brain areas known to play a role in the modulation of emotional processes (Anton et al., 1996) has led several groups to investigate the effects of central administration of nociceptin/orphanin FQ in anxiety models. The neuropeptide was found to exert anxiolytic-like action in the elevated plus-maze and light/dark tests (Jenck et al., 1997) and prevented stress-induced anorexia (Ciccocioppo et al., 2001). In the Mouse Defense Test Battery, nociceptin/orphanin FQ attenuated some, but not all, defensive behaviors, thereby confirming that it may modulate emotional behaviors (Table 3). Risk assessment measures remained unaffected by nociceptin/orphanin FQ, and flight was only decreased at a dose that did also reduce locomotor activity, indicating a nonspecific action of the peptide. In contrast, nociceptin/orphanin FQ clearly and specifically attenuated defensive aggression. The finding that nociceptin/orphanin FQ had positive effects only on terminal defense reactions, displayed when stressful stimuli are unavoidable, suggests that the nociceptin/orphanin FQ system is activated primarily in highly stressful situations. Whether this may indicate that the nociceptin/orphanin FQ system may play a role in the adaptive responses to unavoidable or extreme stress stimuli remains to be established. However, this idea would be in agreement with studies showing that nociceptin/orphanin FQ deficient mice displayed anxiolytic-like activity in stressed animals only (Jenck et al., 2000; Köster et al., 1999).

6.7. Effects of the corticotropin release-inhibiting factor prepro-thyrotropin-releasing hormone$_{178-199}$ in the Mouse Defense Test Battery

Prepro-thyrotropin-releasing hormone$_{178-199}$ (prepro-TRH$_{178-199}$), an intervening peptide of TRH prohormone, has corticotropin release-inhibiting properties, inhibiting both basal and stress-stimulated ACTH secretion in the anterior pituitary (Redei et al., 1995a,b). The peptide has also been localized in several brain areas that are not linked directly to control of pituitary hormone secretion, such as the periaqueductal gray and the lateral septum (Liao et al., 1988). These observations led to the suggestion that prepro-TRH$_{178-199}$ might modulate behavioral reactions to stress. The idea was confirmed by several findings, which showed that the peptide produced anxiolytic-like activity in several classical anxiety tests, including the light/dark and elevated plus-maze tests (McGivern et al., 1997; Stahl et al., 2002). However, it is important to note that the effects displayed by prepro-TRH$_{178-199}$ in these procedures were weak and not dose-dependent. When tested in the Mouse Defense Test Battery at the same dose range and administration route as in these studies, prepro-TRH$_{178-199}$ was devoid of activity on all defensive behaviors (Table 3). The reason for this difference is unclear, but it is worth mentioning that the peptide was also inactive in the light/dark and elevated plus-maze tests and several other anxiety models in our laboratory (unpublished data), thereby questioning the validity of the weak anxiolytic-like effects observed in previous studies.

6.8. Effects of the immunomodulator tuftsin in the Mouse Defense Test Battery

The idea that immunoregulators may play a role in the nervous system is now widely accepted (Plata Salaman, 1991). Particular attention has been paid to the tetrapeptide tuftsin and its analogs, which, in addition to their action on the immune system, have been demonstrated to modulate brain monoaminergic systems (Siemion and Kluczyk, 1999). Notably, it was found that tuftsin normalized dopamine, noradrenaline, and 5-HT contents in several brain areas following stress (Klusha et al., 1987; Semenova et al., 1989; Seredenin et al., 1995). More importantly, tuftsin and its heptapeptide analogue (Thr-Lys-Pro-Arg-Pro-Gly-Pro) displayed comparable anxiolytic-like activity to benzodiazepines in several exploration tests and in a conflict procedure, respectively (Nader and Barrett, 1989; Seredenin et al., 1998). The profile displayed by tuftsin in the Mouse Defense Test Battery is in agreement with these latter findings as the peptide attenuated clearly all defensive behaviors without decreasing locomotor activity (Table 3), thereby showing benzodiazepine-like anxiolytic activity in this model. Although the exact mechanisms underlying these effects remain largely unknown, they involve probably a modulatory action of monoaminergic systems. Future studies will hopefully investigate further the anxiety-reducing potential of tuftsin as it may represent an innovative target for the development of new anxiolytics.

7. Summary

The Mouse Defense Test Battery was specifically developed on the basis of previous rat defense test batteries. These, the Fear/Defense Test Battery and Anxiety/Defense Test Battery, had also provided information on specific defense effects of a number of anxiolytic or potentially anxiolytic drugs, effects that could be, and overwhelmingly have been, confirmed by results of Mouse Defense Test Battery drug studies. Analyses of the behaviors measured in the Mouse Defense Test Battery suggest four factors, each potentially relating to particular aspects of defensiveness. The differential responsivity of these factors to drugs effective against generalized anxiety disorder or panic disorder indicates that the Mouse Defense Test Battery is a specific and appropriate tool for preclinical drug testing for these conditions and raises the possibility of additional links to particular types or aspects of anxiety. The Mouse Defense Test Battery may, therefore, have unusual heuristic utility, suggesting specific questions about the behavioral phenomenology of human anxiety disorders, and providing a behavioral basis for implicating particular brain systems in their expression.

References

Aguilera, G., Rabadan-Diehl, C., 2000. Vasopressinergic regulation of the hypothalamic–pituitary–adrenal axis: implications for stress adaptation. Regulatory Pept. 96, 23–29.

Anton, B., Fein, J., To, T., Li, X., Silberstein, L., Evans, C.J., 1996. Immunohistochemical localization of ORL-1 in the central nervous system of the rat. J. Comp. Neurol. 368, 229–251.

Apter, J.T., Allen, L.A., 1999. Buspirone: future directions. J. Clin. Psychopharmacol. 19, 86–93.

Ashok Raj, B., Sheehan, D.V., 1995. Somatic treatment strategies in panic disorder. In: Asnis, G.M., Van Praag, H.M. (Eds.), Panic Disorder. Clinical, Biological, and Treatment Aspects. Wiley, New York, pp. 279–313.

Barradell, L.B., Fitton, A., 1996. Tandospirone. CNS Drugs 5, 147–153.

Bell, C.J., Nutt, D.J., 1998. Serotonin and panic. Br. J. Psychiatry 172, 465–471.

Belzung, C., Misslin, R., Vogel, E., 1990. Anxiogenic effects of a benzodiazepine receptor partial inverse agonist, RO 19-4603, in a light/dark choice situation. Pharmacol. Biochem. Behav. 36, 593–596.

Belzung, C., Leguisquet, A.M., Crestani, F., 2000. Flumazenil induces benzodiazepine partial agonist-like effects in BALB/c but not C57BL/6 mice. Psychopharmacology 148, 24–32.

Betancur, C., Azzi, M., Rostene, W., 1997. Nonpeptide antagonists of neuropeptide receptors: tools for research and therapy. Trends Pharmacol. Sci. 18, 372–386.

Billett, E.A., Richter, M.A., King, N., Heils, A., Lesch, K.P., Kennedy, J.L., 1997. Obsessive compulsive disorder, response to serotonin reuptake inhibitors and the serotonin transporter gene. Mol. Psychiatry 2, 403–406.

Blanchard, D.C., 1997. Stimulus and environmental control of defensive behaviors. In: Bouton, M., Fanselow, M. (Eds.), The Functional Behaviorism of Robert C. Bolles: Learning, Motivation and Cognition. American Psychological Association, Washington, DC, pp. 283–305.

Blanchard, R.J., Blanchard, D.C., 1972. Effects of hippocampal lesions on the rat's reaction to a cat. J. Comp. Physiol. Psychol. 78, 77–82.

Blanchard, R.J., Blanchard, D.C., 1989. Anti-predator defensive behaviors in a visible burrow system. J. Comp. Psychol. 103, 70–82.

Blanchard, D.C., Rodgers, R.J., Hori, K., Hendrie, C.A., 1988. "Taming" of wild rats (*Rattus rattus*) by 5-HT_{1A} agonists buspirone and gepirone. Pharmacol. Biochem. Behav. 31, 269–278.

Blanchard, D.C., Rodgers, R.J., Hori, K., Hendrie, C.A., Blanchard, R.J., 1989a. Attenuation of defensive threat and attack in wild rats (*Rattus rattus*) by benzodiazepines. Psychopharmacology 97, 392–401.

Blanchard, D.C., Hori, K., Rodgers, R.J., Hendrie, C.A., Blanchard, R.J., 1989b. Differential effects of $5HT_{1A}$ agonists and benzodiazepines on defensive patterns in wild *Rattus rattus*. In: Bevan, P., Cools, A.R., Archer, T. (Eds.), Behavioral Pharmacology of 5-HT. Lawrence Earlbaum, New York, NY, pp. 145–149.

Blanchard, D.C., Blanchard, R.J., Tom, P., Rodgers, R.J., 1990a. Diazepam alters risk assessment in an anxiety/defense test battery. Psychopharmacology 101, 511–518.

Blanchard, D.C., Blanchard, R.J., Rodgers, R.J., 1990b. Pharmacological and neural control of anti-predator defense in the rat. Aggress. Behav. 16, 165–176.

Blanchard, D.C., Blanchard, R.J., Rodgers, R.J., 1991a. Risk assessment and animal models of anxiety. In: Olivier, B., Mos, J., Slangen, J.L. (Eds.), Animal Models in Psychopharmacology. Birkhäuser Verlag, Basel, pp. 117–134.

Blanchard, D.C., Weatherspoon, A., Shepard, J.K., Rodgers, R.J., Blanchard, R.J., 1991b. "Paradoxical" effects of morphine on anti-predator defense reactions in wild and laboratory (*R. norvegicus*) rats. Pharmacol. Biochem. Behav. 40, 819–828.

Blanchard, D.C., Shepherd, J.K., Rodgers, R.J., Blanchard, R.J., 1992. Evidence for differential effects of 8-OH-DPAT on male and female rats in the Anxiety/Defense Test Battery. Psychopharmacology 106, 531–539.

Blanchard, D.C., Shepard, J.K., Rodgers, R.J., Blanchard, R.J., 1993a. Attenuation for antipredator defensive behavior following chronic treatment with the tricyclic antidepressant, imipramine. Psychopharmacology 110, 245–253.

Blanchard, R.J., Yudko, E.B., Rodgers, R.J., Blanchard, D.C., 1993b. Defense system psychopharmacology: an ethological approach to the pharmacology of fear and anxiety. Behav. Brain Res. 58, 155–165.

Blanchard, R.J., Parmigiani, S., Agullana, R., Weiss, S.M., Blanchard, D.C., 1995a. Behaviors of Swiss–Webster and C57 mice in a Fear/Defense Test Battery. Aggress. Behav. 21, 21–28.

Blanchard, R.J., Parmigiani, S., Bjornson, D., Masuda, C., Weiss, S.M., Blanchard, D.C., 1995b. Antipredator behavior of Swiss–Webster mice in a Visible Burrow System. Aggress. Behav. 21, 123–136.

Blanchard, R.J., Griebel, G., Guardiola-Lemaitre, B., Brush, M.M., Lee, J., Blanchard, D.C., 1997a. An ethopharmacological analysis of selective activation of 5-HT1A receptors: the mouse 5-HT1A syndrome. Pharmacol. Biochem. Behav. 57, 897–908.

Blanchard, R.J., Griebel, G., Henrie, J.A., Blanchard, D.C., 1997b. Differentiation of anxiolytic and panicolytic drugs by effects on rat and mouse defense test batteries. Neurosci. Biobehav. Rev. 21, 783–789.

Blanchard, D.C., Griebel, G., Blanchard, R.J., 2001. Mouse defensive behaviors: pharmacological and behavioral assays for anxiety and panic. Neurosci. Biobehav. Rev. 25, 205–218.

Bradwejn, J., Koszycki, D., Couëtoux du Tertre, A., Van Megen, H., Den Boer, J., Westenberg, H., Annable, L., 1994. The panicogenic effects of cholecystokinin-tetrapeptide are antagonized by L-365,260, a central cholecystokinin receptor antagonist, in patients with panic disorder. Arch. Gen. Psychiatry 51, 486–493.

Bradwejn, J., Koszycki, D., Paradis, M., Reece, P., Hinton, J., Sedman, A., 1995. Effect of CI-988 on cholecystokinin tetrapeptide-induced panic symptoms in healthy volunteers. Biol. Psychiatry 38, 742–746.

Broocks, A., Pigott, T.A., Hill, J.L., Canter, S., Grady, T.A., L'Heureux, F., Murphy, D.L., 1998. Acute intravenous administration of ondansetron and m-CPP, alone and in combination, in patients with obsessive–compulsive disorder (OCD): behavioral and biological results. Psychiatry Res. 79 (1), 11–20.

Buller, R., 1995. Reversible inhibitors of monoamine oxidase A in anxiety disorders. Clin. Neuropharmacol. 18, S38–S44.

Calo, G., Bigoni, R., Rizzi, A., Guerrini, R., Salvadori, S., Regoli, D., 2000a. Nociceptin/orphanin FQ receptor ligands. Peptides 21, 935–947.

Calo, G., Guerrini, R., Rizzi, A., Salvadori, S., Regoli, D., 2000b. Pharmacology of nociceptin and its receptor: a novel therapeutic target. Br. J. Pharmacol. 129, 1261–1283.

Cao, B.J., Rodgers, R.J., 1997a. Anxiolytic-like profile of *p*-MPPI, a novel $5HT_{1A}$ receptor antagonist, in the murine elevated plus-maze. Psychopharmacology 129, 365–371.

Cao, B.J., Rodgers, R.J., 1997b. Influence of 5-HT1A receptor antagonism on plus-maze behaviour in mice: 1. Pindolol enantiomers and pindobind 5-HT1A. Pharmacol. Biochem. Behav. 58, 583–591.

Cao, B.J., Rodgers, R.J., 1997c. Influence of 5-HT_{1A} receptor antagonism on plus-maze behaviour in mice: II. WAY 100635, SDZ 216-525 and NAN-190. Pharmacol. Biochem. Behav. 58, 593–603.

Ceulemans, D.L., Hoppenbrouwers, M.L., Gelders, Y.G., Reyntjens, A.J., 1985. The influence of ritanserin, a serotonin antagonist, in anxiety disorders: a double-blind placebo-controlled study versus lorazepam. Pharmacopsychiatry 18, 303–305.

Chalmers, D.T., Lovenberg, T.W., De Souza, E.B., 1995. Localization of novel corticotropin-releasing factor receptor (CRF_2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF_1 receptor mRNA expression. J. Neurosci. 15, 6340–6350.

Chalmers, D.T., Lovenberg, T.W., Grigoriadis, D.E., Behan, D.P., De Souza, E.B., 1996. Corticotrophin-releasing factor receptors: from molecular biology to drug design. Trends Pharmacol. Sci. 17, 166–172.

Ciccocioppo, R., Martin-Fardon, R., Weiss, F., Massi, M., 2001. Nociceptin/orphanin FQ inhibits stress- and CRF-induced anorexia in rats. NeuroReport 12, 1145–1149.

Cutler, N.R., Sramek, J.J., Keppel Hesselink, J.M., Krol, A., Roeschen, J., Rickels, K., Schweizer, E., 1993. A double-blind, placebo-controlled study comparing the efficacy and safety of ipsapirone versus lorazepam in patients with generalized anxiety disorder: a prospective multicenter trial. J. Clin. Psychopharmacol. 13, 429–437.

Darragh, A., Lambe, R., O'Boyle, C., Kenny, M., Brick, I., 1983. Absence of central effects in man of the benzodiazepine antagonist Ro 15-1788. Psychopharmacology 80, 192–195.

Deakin, J.F.W., Graeff, F.G., 1991. 5-HT and mechanisms of defense. J. Psychopharmacol. 5, 305–315.

De Catanzaro, D., 1988. Effect of predator exposure upon early pregnancy in mice. Physiol. Behav. 43, 691–696.

Den Boer, J.A., Westenberg, H.G., 1990. Serotonin function in panic disorder: a double blind placebo controlled study with fluvoxamine and ritanserin. Psychopharmacology 102, 85–94.

Den Boer, J.A., Westenberg, H.G., De Leeuw, A.S., van Viliet, I.M., 1995. Biological dissection of anxiety disorders: the clinical role of selective serotonin reuptake inhibitors with particular reference to fluvoxamine. Int. Clin. Psychopharmacol. 4, 47–52.

Depoortere, H., Zivkovic, B., Lloyd, K.G., Sanger, D.J., Perrault, G., Langer, S.Z., Bartholini, G., 1986. Zolpidem, a novel non-benzodiazepine hypnotic: I. Neuropharmacological and behavioral effects. J. Pharmacol. Exp. Ther. 237, 649–658.

De Vries, G.J., Buijs, R.M., 1983. The origin of the vasopressinergic and oxytocinergic innervation of the rat brain with special reference to the lateral septum. Brain Res. 273, 307–317.

De Weerth, A., Pisegna, J.R., Huppi, K., Wank, S.A., 1993. Molecular cloning, functional expression and chromosomal localization of the human cholecystokinin type A receptor. Biochem. Biophys. Res. Commun. 194, 811–818.

DSM-IV, 1994. Diagnostic and Statistical Manual of Mental Disorders, 4th ed. American Psychiatric Association, Washington, DC.

Duka, T., Ackenheil, M., Noderer, J., Doenicke, A., 1986. Changes in noradrenaline plasma levels and behavioural responses induced by benzodiazepine agonists with the benzodiazepine antagonist Ro 15-1788. Psychopharmacology 90, 351–357.

Dunlop, J., 1998. CCK receptor antagonists. Gen. Pharmacol. 31, 519–524.

Edmunds, M., 1974. Defence in Animals. Longman, New York.

Eisenberg, J.F., 1981. The Mammalian Radiations: An Analysis of Trends in Evolution, Adaptation, and Behavior. University of Chicago Press, Chicago.

Eison, A.S., Eison, M.S., 1994. Serotonergic mechanisms in anxiety. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 18 (1), 47–62.

Elde, R., Schalling, M., Ceccatelli, S., Nakanishi, S., Hokfelt, T., 1990. Localization of neuropeptide receptor mRNA in rat brain: initial observations using probes for neurotensin and substance P receptors. Neurosci. Lett. 120, 134–138.

Endler, J.A., 1986. Defense against predators. In: Feder, M.E., Lauder, G.V. (Eds.), Predator–Prey Relationships. University of Chicago Press, Chicago, pp. 109–134.

Estes, W.K., Skinner, B.F., 1941. Some quantitative properties of anxiety. J. Exp. Psychol. 29, 390–400.

Fichtner, C.G., Poddig, B.E., deVito, R.A., 1997. Post-traumatic stress disorder: pathophysiological aspects and pharmacological approaches to treatment. CNS Drugs 8, 293–322.

Freud, S., 1930. Inhibitions, Symptoms, and Anxiety. Hogarth Press, London.

Fulton, B., Brogden, R.N., 1997. Buspirone: an updated review of its clinical pharmacology and therapeutic applications. CNS Drugs 7, 68–88.

Garakani, H., Zitrin, C.M., Klein, D.F., 1984. Treatment of panic disorder with imipramine alone. Am. J. Psychiatry 141, 446–448.

Giusti, P., Ducic, I., Puia, G., Arban, R., Walser, A., Guidotti, A., Costa, E., 1993. Imidazenil: a new partial positive allosteric modulator of gamma-aminobutyric acid (GABA) action at $GABA_A$ receptors. J. Pharmacol. Exp. Ther. 266, 1018–1028.

Graeff, F.G., 1990. Brain defense system and anxiety. In: Burrows, G.D., Roth, M., Noyes Jr., R. (Eds.), Handbook of Anxiety. The Neurobiology of Anxiety, vol. 3. Elsevier, Amsterdam, pp. 307–343.

Gregory, R.E., Ettinger, D.S., 1998. 5-HT_3 receptor antagonists for the prevention of chemotherapy-induced nausea and vomiting: a comparison of their pharmacology and clinical efficacy. Drug 55 (2), 173–189.

Griebel, G., 1995. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol. Ther. 65, 319–395.

Griebel, G., 1997. Serotonergic drugs in animal models of anxiety: an update. Serotonin ID Res. Alert 2, 251–257.

Griebel, G., 1999a. 5-HT_{1A} receptor blockers as potential drug candidates for the treatment of anxiety disorders. Drug News Perspect. 12, 484–490.

Griebel, G., 1999b. Is there a future for neuropeptide receptor ligands in the treatment of anxiety disorders? Pharmacol. Ther. 82, 1–61.

Griebel, G., Blanchard, D.C., Agnes, R.S., Blanchard, R.J., 1995a. Differential modulation of antipredator defensive behavior in Swiss–Webster mice following acute or chronic administration of imipramine and fluoxetine. Psychopharmacology 120, 57–66.

Griebel, G., Blanchard, D.C., Jung, A., Blanchard, R.J., 1995b. A model of 'antipredator' defense in Swiss–Webster mice: effects of benzodiazepine receptor ligands with different intrinsic activities. Behav. Pharmacol. 6, 732–745.

Griebel, G., Blanchard, D.C., Jung, A., Lee, J.C., Masuda, C.K., Blanchard, R.J., 1995c. Further evidence that the Mouse Defense Test Battery is useful for screening anxiolytic and panicolytic drugs: effects of acute and chronic treatment with alprazolam. Neuropharmacology 34, 1625–1633.

Griebel, G., Blanchard, D.C., Jung, A., Masuda, C.K., Blanchard, R.J., 1995d. 5-HT_{1A} agonists modulate mouse antipredator defensive behavior differently from the 5-HT_{2A} antagonist pirenperone. Pharmacol. Biochem. Behav. 51, 235–244.

Griebel, G., Blanchard, D.C., Blanchard, R.J., 1996a. Evidence that the behaviors in the Mouse Defense Test Battery relate to different emotional states: a factor analytic study. Physiol. Behav. 60, 1255–1260.

Griebel, G., Blanchard, D.C., Blanchard, R.J., 1996b. Predator-elicited flight responses in Swiss–Webster mice: an experimental model of panic attacks. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 20, 185–205.

Griebel, G., Blanchard, D.C., Rettori, M.C., Guardiola-Lemaître, B., Blanchard, R.J., 1996c. Preclinical profile of the mixed 5-HT_{1A}/5-HT_{2A} receptor antagonist S21357. Pharmacol. Biochem. Behav. 54, 509–516.

Griebel, G., Sanger, D.J., Perrault, G., 1996d. The Mouse Defense Test Battery: evaluation of the effects of non-selective and BZ-1 (ω1) selective, benzodiazepine receptor ligands. Behav. Pharmacol. 7, 560–572.

Griebel, G., Perrault, G., Sanger, D.J., 1997a. A comparative study of the effects of selective and non-selective 5-HT2 receptor subtype antagonists in rat and mouse models of anxiety. Neuropharmacology 36, 793–802.

Griebel, G., Perrault, G., Sanger, D.J., 1997b. Behavioural profiles of the reversible monoamine-oxidase-A inhibitors befloxatone and moclobemide in an experimental model for screening anxiolytic and anti-panic drugs. Psychopharmacology 131, 180–186.

Griebel, G., Perrault, G., Sanger, D.J., 1997c. CCK receptor antagonists in animal models of anxiety: comparison between exploration tests, conflict procedures and a model based on defensive behaviours. Behav. Pharmacol. 8, 549–560.

Griebel, G., Curet, O., Perrault, G., Sanger, D.J., 1998a. Behavioral effects of phenelzine in an experimental model for screening anxiolytic and anti-panic drugs: correlation with changes in monoamine-oxidase activity and monoamine levels. Neuropharmacology 37, 927–935.

Griebel, G., Perrault, G., Sanger, D.J., 1998b. Characterization of the behavioral profile of the non peptide CRF receptor antagonist CP-154,526 in anxiety models in rodents—comparison with diazepam and buspirone. Psychopharmacology 138, 55–66.

Griebel, G., Perrault, G., Sanger, D.J., 1998c. Limited anxiolytic-like ef-

fects of non-benzodiazepine hypnotics in rodents. J. Psychopharmacol. 12, 356–365.
Griebel, G., Perrault, G., Sanger, D.J., 1999a. Differences in anxiolytic-like profile of two novel nonbenzodiazepine BZ (ω) receptor agonists on defensive behaviors of mice. Pharmacol. Biochem. Behav. 62, 689–694.
Griebel, G., Perrault, G., Sanger, D.J., 1999b. Orphanin FQ, a novel neuropeptide with anti-stress-like activity. Brain Res. 836, 221–224.
Griebel, G., Perrault, G., Tan, S., Schoemaker, H., Sanger, D.J., 1999c. Pharmacological studies on synthetic flavonoids: comparison with diazepam. Neuropharmacology 38, 965–977.
Griebel, G., Rodgers, R.J., Perrault, G., Sanger, D.J., 1999d. Behavioural profiles in the mouse defence test battery suggest anxiolytic potential of 5-HT_{1A} receptor antagonists. Psychopharmacology 144, 121–130.
Griebel, G., Perrault, G., Sanger, D.J., 2000a. Subtype-selective benzodiazepine receptor ligands. In: Briley, M., Nutt, D. (Eds.), Anxiolytics. Birkhäuser, Basel, pp. 77–94.
Griebel, G., Rodgers, R.J., Perrault, G., Sanger, D.J., 2000b. The effects of compounds varying in selectivity as 5-HT1A receptor antagonists in three rat models of anxiety. Neuropharmacology 39, 1848–1857.
Griebel, G., Moindrot, N., Aliaga, C., Simiand, J., Soubrie, P., 2001a. Characterization of the profile of neurokinin-2 and neurotensin receptor antagonists in the mouse defense test battery. Neurosci. Biobehav. Rev. 25, 619–626.
Griebel, G., Perrault, G., Simiand, J., Cohen, C., Granger, P., Decobert, M., Francon, D., Avenet, P., Depoortere, H., Tan, S., Oblin, A., Schoemaker, H., Evanno, Y., Sevrin, M., George, P., Scatton, B., 2001b. SL651498: an anxioselective compound with functional selectivity for α2- and α3-containing γ-aminobutyric $acid_A$ ($GABA_A$) receptors. J. Pharmacol. Exp. Ther. 298, 753–768.
Griebel, G., Perrault, G., Soubrie, P., 2001c. Effects of SR48968, a selective non-peptide NK2 receptor antagonist on emotional processes in rodents. Psychopharmacology 158, 241–251.
Griebel, G., Simiand, J., Serradeil-Le Gal, C., Wagnon, J., Pascal, M., Scatton, B., Maffrand, J.-P., Soubrié, P., 2002a. Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V1b receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. Proc. Natl. Acad. Sci. U. S. A. 99, 6370–6375.
Griebel, G., Simiand, J., Steinberg, R., Jung, M., Gully, D., Roger, P., Geslin, M., Scatton, B., Maffrand, J.-P., Soubrié, P., 2002b. SSR125 543A: a potent and selective corticotrophin-releasing $factor_1$ receptor antagonist: II. Characterization in rodent models of stress-related disorders. J. Pharmacol. Exp. Ther. 301, 333–345.
Gudelsky, G.A., Berry, S.A., Meltzer, H.Y., 1989. Neurotensin activates tuberoinfundibular dopamine neurons and increases serum corticosterone concentrations in the rat. Neuroendocrinology 49, 604–609.
Gully, D., Canton, M., Boigegrain, R., Jeanjean, F., Molimard, J.C., Poncelet, M., Gueudet, C., Heaulme, M., Leyris, R., Brouard, A., Pelaprat D., Labbé-Jullié, C., Mazella, J., Soubrié, P., Maffrand, J.P., Rostène W., Kitabgi, P., Le Fur, G., 1993. Biochemical and pharmacological profile of a potent and selective nonpeptide antagonist of the neurotensin receptor. Proc. Natl. Acad. Sci. U. S. A. 90, 65–69.
Gutman, D.A., Owens, M.J., Nemeroff, C.B., 2000. Corticotropin-releasing factor antagonists as novel psychotherapeutics. Drugs Future 25, 923–931.
Haefely, W., Martin, J.R., Schoch, P., 1990. Novel anxiolytics that acts as partial agonists at benzodiazepine receptors. Trends Pharmacol. Sci. 11, 452–456.
Helmreich, D.L., Watkins, L.R., Deak, T., Maier, S.F., Akil, H., Watson, S.J., 1999. The effect of stressor controllability on stress-induced neuropeptide mRNA expression within the paraventricular nucleus of the hypothalamus. J. Neuroendocrinol. 11, 121–128.
Hokfelt, T., Skirboll, L., Everitt, B., Meister, B., Brownstein, M., Jacobs T., Faden, A., Kuga, S., Goldstein, M., Markstein, R., 1985. Distribution of cholecystokinin-like immunoreactivity in the nervous system. Co-existence with classical neurotransmitters and other neuropeptides. Ann. N.Y. Acad. Sci. 448, 255–274.
Holsboer, F., 1999. The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. J. Psychiat. Res. 33, 181–214.
Humble, M., Asberg-Wistedt, A., Wistedt, B., Bertilsson, L., 1986. A pilot study of ritanserin (a selective serotonin-2 receptor antagonist) in panic disorder and agoraphobia. Clinical and biochemical effects. C.I.N.P. Congress, Puerto Rico, 165.
Jacobson, A.F., Dominguez, R.A., Goldstein, B.J., Steinbook, R.M., 1985. Comparison of buspirone and diazepam in generalized anxiety disorder. Pharmacotherapy 5, 290–296.
Jenck, F., Moreau, J.L., Martin, J.R., Kilpatrick, G.J., Reinscheid, R.K., Monsma, F.J., Nothacker, H.P., Civelli, O., 1997. Orphanin FQ acts as an anxiolytic to attenuate behavioral responses to stress. Proc. Natl. Acad. Sci. U. S. A. 94, 14854–14858.
Jenck, F., Ouagazzal, A.M., Pauly Evers, M., Moreau, J.L., 2000. Orphanin FQ: role in behavioral fear responses and vulnerability to stress? Mol. Psychiatry 5, 572–574.
Kay, B., Healy, T.E., 1984. Premedication by controlled-release morphine. Anaesthesia 39 (6), 587–589.
King, S.M., 1999. Escape-related behaviours in an unstable elevated and exposed environment: I. A new behavioural model of extreme anxiety. Behav. Brain Res. 98, 113–126.
Klusha, V.E., Mutsenietse, R.K., Svirskis, S.V., Zalitis, G.M., Liepa, I.R., 1987. Neuroimmunoregulatory properties of short protein fragments in rats exposed to immobilization stress. Bull. Eksp. Biol. Med. 104, 186–187.
Köster, A., Montkowski, A., Schulz, S., Stube, E.M., Knaudt, K., Jenck, F., Moreau, J.L., Nothacker, H.P., Civelli, O., Reinscheid, R.K., 1999. Targeted disruption of the orphanin FQ/nociceptin gene increases stress susceptibility and impairs stress adaptation in mice. Proc. Natl. Acad. Sci. U. S. A. 96, 10444–10449.
Kramer, M.S., Cutler, N.R., Ballenger, J.C., Patterson, W.M., Mendels, J., Chenault, A., Shrivastava, R., Matzurawolfe, D., Lines, C., Reines, S., 1995. A placebo-controlled trial of L-365,260, a CCK_B antagonist, in panic disorder. Biol. Psychiatry 37, 462–466.
Lader, M., 1994. Benzodiazepines: a risk–benefit profile. CNS Drugs 1, 377–387.
Leyhausen, P., 1969. Cat Behavior: The Predatory and Social Behavior of Domestic and Wild Cats. Garland STPM Press, New York, 1979.
Liao, N., Bulant, M., Nicholas, P., Vaudry, H., Pelletier, G., 1988. Immunocytochemical distribution of neurons containing a peptide derived from thyrotropin-releasing hormone precursor in the rat brain. Neurosci. Lett. 85, 24–28.
Liebowitz, M.R., Hollander, E., Schneier, F., Campeas, R., Welkowitz, L., Hatterer, J., Fallon, B., 1990. Reversible and irreversible monoamine oxidase inhibitors in other psychiatric disorders. Acta Psychiatr. Scand., Suppl. 360, 29–34.
Lines, C., Challenor, J., Traub, M., 1995. Cholecystokinin and anxiety in normal volunteers: an investigation of the anxiogenic properties of pentagastrin and reversal by the cholecystokinin receptor subtype B antagonist L-365,260. Br. J. Clin. Pharmacol. 39, 235–242.
Lolait, S.J., O'Carroll, A.M., Mahan, L.C., Felder, C.C., Button, D.C., Young III, W.S., Mezey, E., Brownstein, M.J., 1995. Extrapituitary expression of the rat V1b vasopressin receptor gene. Proc. Natl. Acad. Sci. U. S. A. 92, 6783–6787.
Low, K., Crestani, F., Keist, R., Benke, D., Brunig, I., Benson, J.A., Fritschy, J.M., Rulicke, T., Bluethmann, H., Mohler, H., Rudolph, U., 2000. Molecular and neuronal substrate for the selective attenuation of anxiety. Science 290, 131–134.
Maddock, R.J., 1998. Acute effects of low-dose flumazenil in panic disorder. J. Clin. Psychopharmacol. 18, 258–261.
Maggi, C.A., 1995. The mammalian tachykinin receptors. Gen. Pharmacol. 26, 911–944.
Martin, J.R., Pieri, L., Bonetti, E.P., Schaffner, R., Burkard, W.P., Cumin R., Haefely, W.E., 1988. Ro 16-6028: a novel anxiolytic acting as a

partial agonist at the benzodiazepine receptor. Pharmacopsychiatry 21, 360–362.
McCann, S.M., Vijayan, E., Koenig, J., Krulich, L., 1982. The effects of neurotensin on anterior pituitary hormone secretion. Ann. N.Y. Acad. Sci. 400, 160–171.
McCarthy, J.R., Heinrichs, S.C., Grigoriadis, D.E., 1999. Recent advances with the CRF1 receptor: design of small molecule inhibitors, receptor subtypes and clinical indications. Curr. Pharm. Des. 5, 289–315.
McGivern, R.F., Rittenhouse, P., Aird, F., Van de Kar, L.D., Redei, E., 1997. Inhibition of stress-induced neuroendocrine and behavioral response in the rat by prepro-thyrotropin-releasing hormone 178–199. J. Neurosci. 17, 4886–4894.
McLeod, D.R., Hoehn-Saric, R., Porges, S.W., Kowalski, P.A., Clark, C.M., 2000. Therapeutic effects of imipramine are counteracted by its metabolite, desipramine, in patients with generalized anxiety disorder. J. Clin. Psychopharmacol. 20 (6), 615–621.
Meunier, J.C., Mollereau, C., Toll, L., Suaudeau, C., Moisand, C., Alvinerie, P., Butour, J.L., Guillemot, J.C., Ferrara, P., Monsarrat, B., 1995. Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor (see comments). Nature 377, 532–535.
Modigh, K., 1987. Antidepressant drugs in anxiety disorders. Acta Psychiatr. Scand., Suppl. 335, 57–74.
Moran, T.H., Robinson, P.H., Goldrich, M.S., McHugh, P.R., 1986. Two brain cholecystokinin receptors: implications for behavioral actions. Brain Res. 362, 175–179.
Morel, A., O'Carroll, A.M., Brownstein, M.J., Lolait, S.J., 1992. Molecular cloning and expression of a rat V1a arginine vasopressin receptor. Nature 356, 523–526.
Nader, M.A., Barrett, J.E., 1989. Effects of corticotropin-releasing factor (CRF), tuftsin and dermorphin on behavior of squirrel monkeys maintained by different events. Peptides 10, 1199–1204.
Nemeroff, C.B., Luttinger, D., Hernandez, D.E., Mailman, R.B., Mason G.A., Davis, S.D., Widerlov, E., Frye, G.D., Kilts, C.A., Beaumont, K., Breese, G.R., Prange Jr., A.J., 1983. Interactions of neurotensin with brain dopamine systems: biochemical and behavioral studies. J. Pharmacol. Exp. Ther. 225, 337–345.
Nicot, A., Berod, A., Gully, D., Rowe, W., Quirion, R., De Kloet, E.R., Rostene, W., 1994. Blockade of neurotensin binding in the rat hypothalamus and of the central action of neurotensin on the hypothalamic–pituitary–adrenal axis with non-peptide receptor antagonists. Neuroendocrinology 59, 572–578.
Nicot, A., Rowe, W.B., De Kloet, E.R., Betancur, C., Jessop, D.S., Lightman, S.L., Quirion, R., Rostene, W., Berod, A., 1997. Endogenous neurotensin regulates hypothalamic–pituitary–adrenal axis activity and peptidergic neurons in the rat hypothalamic paraventricular nucleus. J. Neuroendocrinol. 9, 263–269.
Nikulina, E.M., 1991. Neural control of predatory aggression in wild and domesticated animals. Neurosci. Biobehav. Rev. 15, 545–547.
Nutt, D.J., Glue, P., Lawson, C., Wilson, S., 1990. Flumazenil provocation of panic attacks: evidence for altered benzodiazepine receptor sensitivity in panic disorder. Arch. Gen. Psychiatry 47, 917–925.
Otsuka, M., Yoshioka, K., 1993. Neurotransmitter functions of mammalian tachykinins. Physiol. Rev. 73, 229–308.
Pande, A.C., 1997. Lack of efficacy of a cholecystokinin-B antagonist in anxiety disorders. Biol. Psychiatry 42, 11–19.
Pecknold, J.C., Luthe, L., Scott Fleury, M.H., Jenkins, S., 1993. Gepirone and the treatment of panic disorder: an open study. J. Clin. Psychopharmacol. 13, 145–149.
Pinel, J.P.J., Mana, M.J., 1989. Adaptive interactions of rats with dangerous inanimate objects: support for a cognitive theory of defensive behavior. In: Blanchard, R.J., Brain, P.F., Blanchard, D.C., Parmigiani, S. (Eds.), Ethoexperimental Approaches to the Study of Behavior. Kluwer Academic Publishers, Dordrecht, pp. 137–151.
Pisegna, J.R., de Weerth, A., Huppi, K., Wank, S.A., 1992. Molecular cloning of the human brain and gastric cholecystokinin receptor: structure, functional expression and chromosomal localization. Biochem. Biophys. Res. Commun. 189, 296–303.
Plata Salaman, C.R., 1991. Immunoregulators in the nervous system. Neurosci. Biobehav. Rev. 15, 185–215.
Pohl, R., Balon, R., Yeragani, V.K., Gershon, S., 1989. Serotonergic anxiolytics in the treatment of panic disorder: a controlled study with buspirone. Psychopathology 22 (Suppl. 1), 60–67.
Pollack, M.H., Rosenbaum, J.F., 1988. Benzodiazepines in panic-related disorders. Special issue: perspectives on panic-related disorders. J. Anxiety Disord. 2, 95–107.
Potokar, J., Nutt, D.J., 1994. Anxiolytic potential of benzodiazepine receptor partial agonists. CNS Drugs 1, 305–315.
Priest, R.G., Gimbrett, R., Roberts, W., Steinert, J., 1995. Reversible and selective inhibitors of monoamine oxidase A in mental and other disorders. Acta Psychiatr. Scand. 91, 40–43.
Redei, E., Hilderbrand, H., Aird, F., 1995a. Corticotropin release-inhibiting factor is encoded within prepro-TRH. Endocrinology 136, 1813–1816.
Redei, E., Hilderbrand, H., Aird, F., 1995b. Corticotropin release-inhibiting factor is prepro-thyrotropin-releasing hormone (178–199). Endocrinology 136, 3557–3563.
Regoli, D., Boudon, A., Fauchere, J.L., 1994. Receptors and antagonists for substance P and related peptides. Pharmacol. Rev. 46, 551–599.
Reinscheid, R.K., Nothacker, H.P., Bourson, A., Ardati, A., Henningsen R.A., Bunzow, J.R., Grandy, D.K., Langen, H., Monsma Jr., F.J., Civelli, O., 1995. Orphanin FQ: a neuropeptide that activates an opioid like G protein-coupled receptor. Science 270, 792–794.
Rickels, K., Schweizer, E., DeMartinis, N., Mandos, L., Mercer, C., 1997. Gepirone and diazepam in generalized anxiety disorder: a placebo-controlled trial. J. Clin. Psychopharmacol. 17, 272–277.
Roberts, G.W., Woodhams, P.L., Polak, J.M., Crow, T.J., 1982. Distribution of neuropeptides in the limbic system of the rat: the amygdaloid complex. Neuroscience 7, 99–131.
Rodgers, R.J., Blanchard, D.C., Wong, L.E., Blanchard, R.J., 1990. Effects of scopolamine on antipredator defense reactions in wild and laboratory rats. Pharmacol. Biochem. Behav. 36, 575–583.
Rowe, W., Viau, V., Meaney, M.J., Quirion, R., 1995. Stimulation of CRH-mediated ACTH secretion by central administration of neurotensin: evidence for the participation of the paraventricular nucleus. J. Neuroendocrinol. 7, 109–117.
Rowe, W.B., Nicot, A., Sharma, S., Gully, D., Walker, C.D., Rostene W.H., Meaney, M.J., Quirion, R., 1997. Central administration of the neurotensin receptor antagonist, SR48892, modulates diurnal and stress-related hypothalamic–pituitary–adrenal activity. Neuroendocrinology 66, 75–85.
Rudolph, U., Crestani, F., Benke, D., Brunig, I., Benson, J.A., Fritschy J.M., Martin, J.R., Bluethmann, H., Möhler, H., 1999. Benzodiazepine actions mediated by specific γ-aminobutyric $acid_A$ receptor subtypes. Nature 401, 796–800.
Russell, J.M., Koran, L.M., Rush, J., Hirschfeld, R.M., Harrison, W., Friedman, E.S., Davis, S., Keller, M., 2001. Effect of concurrent anxiety on response to sertraline and imipramine in patients with chronic depression. Depress. Anxiety 13 (1), 18–27.
Sarret, P., Beaudet, A., Vincent, J.P., Mazella, J., 1998. Regional and cellular distribution of low affinity neurotensin receptor mRNA in adult and developing mouse brain. J. Comp. Neurol. 394, 344–356.
Schopf, J., Laurian, S., Le, P.K., Gaillard, J.M., 1984. Intrinsic activity of the benzodiazepine antagonist Ro 15-1788 in man: an electrophysiological investigation. Pharmacopsychiatry 17, 79–83.
Semenova, G.P., Gurevich, E.V., Kozlovskaia, M.M., Gromova, E.A., 1989. The role of the brain monoaminergic systems in the effects of tuftsin and its analog on animal emotional behavior. Fiziol. Z. SSSR 75, 759–765.
Seredenin, S.B., Semenova, T.P., Kozlovskaia, M.M., Medvinskaia, N.I., Nezovibatko, V.N., 1995. The characteristics of the anxiolytic action of tuftsin and its analog TP-7 on behavior and serotonin metabolism in the brain of rats with chronic deprivation of serotoninergic system activity. Eksp. Klin. Farmakol. 58, 3–6.

Seredenin, S.B., Kozlovskaya, M.M., Blednov, Y.A., Semenova, T.P., Kozlovskii, I.I., CzabakGarbacz, R., Nezavibatko, V.N., Myasoedov, N.F., 1998. The study of anxiolytic activity of tuftsin analogue in inbred mice with different types of emotional-stress reaction. Z. Vyss. Nervn. Deat. Pavlova 48, 153–160.

Serradeil-Le Gal, C., Wagnon, J., Simiand, J., Griebel, G., Lacour, C., Guillon, G., Barberis, C., Brossard, G., Soubrié, P., Nisato, D., Pascal, M., Pruss, R., Scatton, B., Maffrand, J.P., Le Fur, G., 2002. Characterization of (2*S*,4*R*)-1-[5-chloro-1-[(2,4-dimethoxyphenyl)sulfonyl]-3-(2-methoxy-phenyl)-2-oxo-2,3-dihydro-1*H*-indol-3-yl]-4-hydroxy-*N*,*N*-dimethyl-2-pyrrolidine carboxamide (SSR149415), a selective and orally active vasopressin V_{1b} receptor antagonist. J. Pharmacol. Exp. Ther. 300, 1122–1130.

Sheehan, D.V., Raj, A.B., Sheehan, K.H., Soto, S., 1990. Is buspirone effective for panic disorder? J. Clin. Psychopharmacol. 10, 3–11.

Shepherd, J.K., Rodgers, R.J., Blanchard, R.J., Magee, L., Blanchard, D.C., 1993. Ondansetron, gender, and antipredator defensive behavior. J. Psychopharmacol. 7, 72–81.

Siemion, I.Z., Kluczyk, A., 1999. Tuftsin: on the 30-year anniversary of Victor Najjar's discovery. Peptides 20, 645–674.

Smith, P.A., Moran, T.D., 2001. The nociceptin receptor as a potential target in drug design. Drug News Perspect. 14, 335–345.

Solyom, L., Solyom, C., Ledwidge, B., 1991. Fluoxetine in panic disorder. Can. J. Psychiatry 36, 378–380.

Squires, R.F., Benson, D.I., Braestrup, C., Coupet, J., Klepner, C.A., Myers, V., Beer, B., 1979. Some properties of brain specific benzodiazepine receptors: new evidence for multiple receptors. Pharmacol. Biochem. Behav. 10, 825–830.

Sramek, J.J., Robinson, R.E., Suri, A., Cutler, N.R., 1995. Efficacy trial of the 5-HT2, antagonist MDL 11,939 in patients with generalized anxiety disorder. J. Clin. Psychopharmacol. 15, 20–22.

Stahl, C.E., Redei, E., Wang, Y., Borlongan, C.V., 2002. Behavioral, hormonal and histological stress markers of anxiety-separation in postnatal rats are reduced by prepro-thyrotropin-releasing hormone 178–199. Neurosci. Lett. 321, 85–89.

Steinberg, R., Alonso, R., Griebel, G., Bert, L., Jung, M., Oury-Donat, F., Poncelet, M., Gueudet, C., Desvignes, C., Le Fur, G., Soubrie, P., 2001. Selective blockade of neurokinin-2 receptors produces antidepressant-like effects associated with reduced corticotropin-releasing factor function. J. Pharmacol. Exp. Ther. 299, 449–458.

St. Pierre, S., Quirion, R., Regoli, D., Gervais, A., Lavigne, F., Poirier, A., Jolicoeur, F., Barbeau, A., Rioux, F., 1980. Neurotensin, a multi-action peptide hormone. Union Med. Can. 109, 1447–1455.

Treves, A., 1999. Vigilance and spatial cohesion among blue monkeys. Folia Primatol. (Basel) 70 (5), 291–294.

Vaccari, C., Lolait, S.J., Ostrowski, N.L., 1998. Comparative distribution of vasopressin V1b and oxytocin receptor messenger ribonucleic acids in brain. Endocrinology 139, 5015–5033.

Vale, W.W., Spiess, J., Rivier, C., Rivier, J., 1981. Characterization of a 41 residue ovine hypothalamic peptide that stimulates the secretion of corticotropin and β-endorphin. Science 213, 1394–1397.

Vale, W.W., Rivier, C., Brown, M.R., Spiess, J., Koob, G., Swanson, L., Bilezikjian, L., Bloom, F., Rivier, J., 1983. Chemical and biological characterization of corticotropin-releasing factor. Recent Prog. Horm. Res. 39, 245–270.

Van Vliet, I.M., Westenberg, H.G.M., Den Boer, J.A., 1996. Effects of the 5-HT1A receptor agonist flesinoxan in panic disorder. Psychopharmacology 127, 174–180.

Walker, N., Lepee-Lorgeoux, I., Fournier, J., Betancur, C., Rostene, W., Ferrara, P., Caput, D., 1998. Tissue distribution and cellular localization of the levocabastine-sensitive neurotensin receptor mRNA in adult rat brain. Brain Res., Mol. Brain Res. 57, 193–200.

Westenberg, H.G.M., 1996. Developments in the drug treatment of panic disorder: what is the place of the selective serotonin reuptake inhibitors? J. Affect. Disord. 40, 85–93.

Westenberg, H.G., Den Boer, J.A., 1989. Selective monoamine uptake inhibitors and a serotonin antagonist in the treatment of panic disorder. Psychopharmacol. Bull. 25, 119–123.

Westenberg, H.G.M., Den Boer, J.A., 1993. Serotonin in anxiety related disorders. In: Vanhoutte, P.M., Saxena, P.R., Paoletti, R., Brunello, N., Jackson, A.S. (Eds.), Serotonin, From Cell Biology to Pharmacology and Therapeutics. Kluwer Academic Publishers, Dordrecht, pp. 249–254.

Westenberg, H.G.M., Van Vliet, I.M., Den Boer, J.A., 1992. Flesinoxan, a selective 5-$HT1_A$ agonist, in the treatment of panic disorder. Clin. Neuropharmacol. 15, 60 (Suppl.).

Woods, J.H., Winger, G., 1995. Current benzodiazepine issues. Psychopharmacology 118, 107–115.

Woods, S.W., Charney, D.S., Silver, J.M., Krystal, J.H., Heninger, G.R., 1991. Behavioral, biochemical, and cardiovascular responses to the benzodiazepine receptor antagonist flumazenil in panic disorder. Psychiatry Res. 36, 115–127.

Young, L.J., Toloczko, D., Insel, T.R., 1999. Localization of vasopressin (V1a) receptor binding and mRNA in the rhesus monkey brain. J. Neuroendocrinol. 11, 291–297.

Zohar, J., Westenberg, H.G.M., 2000. Anxiety disorders: a review of tricyclic antidepressants and selective serotonin reuptake inhibitors. Acta Psychiatr. Scand. 101, 39–49.