ELSEVIER

Physiology & Behavior, Vol. 60, No. 5, pp. 1255–1260, 1996

0031-9384/96 $15.00 + .00

PII S0031-9384(96)00230-2

Evidence that the Behaviors in the Mouse Defense Test Battery Relate to Different Emotional States: A Factor Analytic Study

GUY GRIEBEL,*[1] D. CAROLINE BLANCHARD† AND ROBERT J. BLANCHARD†

*CNS Research Department, Synthélabo Recherche, 31, avenue Paul-Vaillant Couturier, 92220 Bagneux, France and
†Békésy Laboratory of Neurobiology, University of Hawaii, 1993 East-West Road, Honolulu, HI 96822 USA

Received 19 March 1996

GRIEBEL, G., D. C. BLANCHARD AND R. J. BLANCHARD. *Evidence that the behaviors in the Mouse Defense Test Battery relate to different emotional states: A factor analytic study.* PHYSIOL BEHAV **60**(5) 1255–1260, 1996.—The Mouse Defense Test Battery (MDTB) has been designed to investigate defensive responses of Swiss-Webster mice confronted with a natural threat, a rat. These behaviors include flight, avoidance, defensive threat/attack reactions, and risk assessment activities. In the present study, a factor analysis was used to examine potential relationships between these behavioral responses. Five independent Factors were extracted from the 17 parameters obtained in the MDTB. Both Factor 1 and Factor 2 include cognitive aspects of defensive behaviors that appear to be related to the process of acquiring and analyzing information in the presence of threatful stimuli (i.e., risk assessment). Flight/avoidance responses heavily loaded on Factor 3 and to a lesser extent on Factor 4. Several defensive threat/attack reactions (i.e., upright postures and biting) highly loaded on Factor 4 and biting also loaded on Factor 3. Finally, the variables that loaded highly on Factor 5 were the number of wall rearings and climbings following the removal of the rat and the immobility time when the subject was trapped in a straight alley. Although the meaning of this latter Factor is not clear at present, Factors 1 to 4 seemingly relate to anxiety. Taken together with recent drug findings from the MDTB, the present analysis further supports the idea that this model provides measures that reflect different aspects of anxiety.

Mouse Defense Test Battery Factor analysis Risk assessment Flight Defensive threat/attack Anxiety Swiss-Webster mouse

THE rodent defense test battery measures a full range of specific defensive behaviors either to a discrete, highly discriminable, and present threat source (e.g., a cat or a rat) or to situations closely associated with the potential for danger but not presenting a discrete, clearly dangerous, stimulus. Primary measures taken comprise escape attempts, freezing, risk assessment (RA), defensive sonic vocalization and attack, and flight. The battery has been validated for use in rats (6) and in mice (11,13). In light of the suggestion that the defensive behaviors of lower mammals constitute a significant model for understanding human emotional disorders (3,5), several studies demonstrated that defense reactions were bidirectionally sensitive to pharmacological manipulations designed to modulate anxiety-related responses (for reviews, see 7,15). For instance, Blanchard and collaborators (7), using laboratory as well as wild rats, demonstrated that benzodiazepines (BZ) (diazepam, chlordiazepoxide, midazolam), 5-HT_{1A} receptor ligands (buspirone, gepirone, 8-OH-DPAT) and alcohol produce a profile of effects primarily involving RA activities and defensive threat/attack responses. Several recent experiments using the murine defense test battery (MDTB) confirmed that defense reactions may be of particular interest for studying potential anxiety-modulating properties of psychoactive drugs (10–13). Moreover, findings from the latter studies showed that, although anxiolytics generally reduced the level of defensiveness, some dissimilarities in drug action were observed depending on the class of compounds used. As an illustration, BZ receptor agonists (chlordiazepoxide, Ro 19-8022) and 5-HT_{1A} receptor ligands (gepirone, 8-OH-DPAT) showed a clear separation of which RA behaviors were affected, with BZ reducing RA to an approaching rat, and the 5-HT compounds reduced RA, again to the rat, but only when the subject was trapped in a straight alley with the rat remaining at a constant distance. Moreover, tests with panicolytics (alprazolam, imipramine, and fluoxetine) indicated that these reduced flight reactions (10,12), which generally were not altered by drugs used in the clinical management of generalized anxiety disorders (chlordiazepoxide, gepirone) (11,13). These latter drugs mainly reduced RA responses, activities that were little affected by panicolytic compounds.

These behavioral commonalities and dissimilarities suggest that particular patterns of drug effects may map rather precisely

[1] To whom requests for reprints should be addressed.

onto the target symptoms for specific psychopathologies. Therefore, the relationships between the variety of responses measured in the defense test battery become an important issue. Do these different responses provide different measures of the same state, or do they measure distinct states of defensiveness, fear, or anxiety? This question can be approached using a factor analysis of the various behavioral defense reactions observed in the battery.

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. The analysis can be conducted either on the behavioral responses measured within a single test, or with parameters from several tests. In a study using mice, Lister (14) subjected the scores from the elevated plus-maze to a factor analysis. He isolated three independent Factors: the first related to anxiety, the second reflecting exploration, and the third motor activity. More recently, two studies (8,16) demonstrated that the addition of a range of ethological-based measures to the elevated plus-maze yielded additional Factors. Among these, several were thought to represent RA. Finally, File (9) and Belzung et al. (1,2) revealed that parameters recorded in several anxiety models (e.g., elevated plus-maze, social interaction, hole-board, Vogel conflict, light/dark, and free-exploration) produced distinct anxiety Factors, thereby indicating that they reflect different emotional states.

In the present study, the application of a factor analytical technique was used to examine potential relationships between the behavioral responses recorded in the MDTB.

METHOD

Animals

The subjects were 80 naive male Swiss-Webster mice obtained from Simonsen Laboratories (CA), 60–75 days old at the beginning of the experiment, and 5 male Long Evans rats (400–500 g) bred in the laboratory. Prior to experimental testing, they were housed singly during 1 week in a standard cage (mice: 30 × 20 × 14 cm; rats: 44 × 30 × 20 cm) containing a constant supply of food pellets and water. All animals were maintained under standard laboratory conditions (21–23°C) and kept on a 12-h light/dark cycle with light onset at 0600 h.

Apparatus

The test was conducted in an oval runway, 0.40 m wide, 0.30 m high, and 4.4 m in total length, consisting of 2 2-m straight segments joined by 2 0.4-m curved segments and separated by a median wall (2.0 × 0.30 × 0.06). The apparatus was elevated to a height of 0.80 m from the floor to enable the experimenter to easily hold the rat, while minimizing the mouse's visual contact with him. All parts of the apparatus were made of black Plexiglas. The floor was marked every 20 cm to facilitate distance measurement. Activity was recorded with videocameras mounted above the apparatus. Twelve to fifteen mice were stressed by the same rat. The experiments were performed under red light between 0900 and 1400 h.

Procedure

Pretest: 3-min familiarization period. The subjects were individually placed into the runway for a 3-min period to allow them to familiarize with the apparatus.

Reactions to the Rat

Rat avoidance test (min 4 to 6). Immediately after the 3-min familiarization period, a hand-held dead rat (killed by CO_2 inhalation just before the experiment was started) was introduced into the runway and brought up to the subject at a speed of approximately 0.5 m/s. Approach was terminated when contact with the subject was made or the subject ran away from the approaching rat. If the subject fled, avoidance distance (the distance from the rat to the subject at the point of flight) was recorded. This was repeated 5 times and mean values of avoidance distance and frequency were calculated.

Chase test (min 7 to 8). The hand-held rat was brought up to the subject at a speed of approximately 2.0 m/s. The following parameters were recorded: Flight speed (measured when the subject is running straight), number of stops (pause in movement), orientations (subject stops, then orients the head toward the rat) and reversals (subject stops, then runs in the opposite direction).

Straight alley test (min 9 to 11). The runway was then converted to a straight alley by the closing of a door at one end. Three approaches, 15 s each, terminating respectively at 1.20, 0.80, and 0.40 m were made by the hand-held rat toward the subject in this inescapable runway. Measures taken included immobility time, closest distance between the subject and the rat, and the number of approaches/withdrawals (subject must move more than 0.2 m forward from the closed door, then return to it). Finally, the experimenter brought the rat up to contact the subject. For each such contact, bites, vocalizations, upright postures, and jump attacks by the subjects were noted. This was repeated 3 times and mean values for each response were calculated.

Posttest: Contextual Defense

Immediately after the straight alley test, the rat was removed and the doors were opened. Line crossings, wall rears, wall climbs, and jump escapes were recorded during a 3-min session (min 12 to 14).

Statistics

A principal component solution and an orthogonal rotation (varimax) of the factor matrix were employed to analyze the data, so that the factors isolated were independent of each other. The Cattell Scree test was additionally used to identify the factor

TABLE 1

BEHAVIORS DISPLAYED BY MICE IN THE MOUSE DEFENSE TEST BATTERY

	Mean ± SEM
Avoidance distance (cm)	114.3 ± 4.09
Avoidance frequency	4.39 ± 0.13
Flight speed (m/s)	1.08 ± 0.03
Reversals	2.21 ± 0.27
Head orientations	3.84 ± 0.34
Stops	10.71 ± 0.52
Approaches/withdrawals	3.49 ± 0.19
Closest distance between animals (cm)	109.1 ± 5.62
Immobility/freezing (s)	6.16 ± 0.81
Vocalizations	2.88 ± 0.06
Upright postures	2.50 ± 0.08
Bitings	2.84 ± 0.07
Jump attacks	1.34 ± 0.12
Line crossings	162.7 ± 3.57
Rearings	7.41 ± 0.55
Wall climbings	15.19 ± 0.81
Jump escapes	2.15 ± 0.38

pattern matrix. The factor loading for each behavioral measure provides an estimate of how well that parameter reflects a particular variable; thus, a value of 1.00 represents a perfect (negative or positive) correlation and a loading of less than 0.40 suggests that a particular parameter is a poor measure of a variable. Consequently, only loadings greater than 0.40 are reported.

RESULTS

Behavioral Profile

Table 1 presents the mean value of each parameter recorded in the MDTB. In the rat avoidance test, subjects showed a clear pattern of flight responses. Of 5 approaches, avoidance reactions occurred predominantly (88%) with an average avoidance distance of 114 cm. During the chase test, mice often interrupted their flight (mean about 10 stops); occasionally oriented toward the oncoming rat (mean about 4 orientations); and sometimes reversed to run in the opposite direction (mean about 2 reversals). When trapped in one part of the runway, subjects displayed a high level of activity (86%) that mainly occurred far from the rat because they stayed distant from the threatening stimulus (110 cm) and because the frequency of approaches toward the rat followed by withdrawal responses were rather low (mean about 3 approaches/withdrawals). Upon forced contact, the defense reactions rank order was as follow: vocalizations (96%) = bitings (95%) > upright postures (83%) > jump attacks (44%). Finally, when the rat was removed, the subjects' activity mainly consisted of horizontal ambulation (87%). Wall climbing represented the main vertical activity (8%), followed by wall rearings (4%), and jump escapes (1%).

Factor Analysis

The correlations between the different behavioral measures are given in Table 2. Seven Factors emerged from the analysis corresponding to those with eigenvalues greater than 1 and these accounted for 75.97% of the variance (Table 3). Each behavioral index loaded on one or another of the 7 Factors. Among these Factors, 5 clearly emerged, accounting for 62.96% of the total variance (Table 4). Factors 6 and 7 showed loadings for only one variable each (i.e., number of avoidances and line crossings, respectively) and were therefore not considered in the analysis. With the exception of the number of jump attacks, variables that saturated Factor 1 were all related to RA activities (i.e., number of reversals, orientations, stops, and approaches/withdrawals). Variables with high loadings on Factor 2 were the number of line crossings and two measures recorded during the straight alley test (i.e., approaches/withdrawals and closest distance between animals). Measures obtained from 4 different test situations (rat avoidance: avoidance distance and frequency; chase: speed; straight alley: closest distance between animals and immobility time; forced contact: bitings) loaded very highly on Factor 3. Two variables obtained during the forced contact test (upright postures and bitings), avoidance frequency, and postrat jump escapes showed a high loading on Factor 4. Finally, wall rearings and climbings and, to a lesser extent, immobility loaded highly on Factor 5.

DISCUSSION

It is important to know about the relationship between measures obtained in the MDTB. Most of the data currently available stem from pharmacological analyses of each particular test phase (10–13). This study used a different approach. It compared the defensive behaviors exhibited by undrugged animals in each phase. The behavioral profile indicated that, in response to an

TABLE 2

CORRELATIONS BETWEEN THE 17 MEASURES RECORDED FROM THE MOUSE DEFENSE TEST BATTERY

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 Avoidance distance	*																
2 Avoidance frequency	0.16	*															
3 Flight speed	0.17	0.27	*														
4 Reversals	0.17	−0.01	0.41	*													
5 Head orientations	−0.03	0.01	0.35	0.73	*												
6 Stops	−0.11	−0.20	−0.03	0.43	0.63	*											
7 Approaches/withdrawals	−0.02	−0.07	0.06	−0.06	−0.27	−0.44	*										
8 Closest distance between animals	0.19	0.24	−0.12	−0.17	−0.06	0.09	−0.58	*									
9 Immobility/freezing	0.00	0.18	0.26	0.07	0.04	−0.10	−0.22	0.30	*								
10 Vocalizations	−0.18	0.02	0.09	0.19	0.23	0.11	0.17	−0.15	−0.22	*							
11 Upright postures	0.18	0.07	−0.03	0.17	0.19	0.03	0.08	0.03	−0.22	0.03	*						
12 Bitings	0.26	0.08	0.01	0.16	−0.20	−0.32	0.22	0.13	0.11	−0.06	0.37	*					
13 Jump attacks	0.10	−0.06	0.15	−0.01	−0.28	−0.28	0.35	−0.19	0.26	−0.15	−0.30	0.26	*				
14 Line crossings	−0.21	−0.09	0.07	0.21	−0.01	−0.08	0.36	−0.41	0.04	0.12	0.10	0.19	0.01	*			
15 Rearings	0.14	0.06	−0.08	−0.18	−0.24	−0.00	−0.05	0.16	−0.15	0.11	−0.11	−0.05	−0.01	0.09	*		
16 Wall climbings	−0.05	−0.11	−0.28	−0.18	−0.08	−0.07	−0.13	−0.03	0.12	−0.13	−0.25	−0.15	−0.17	−0.26	−0.32	*	
17 Jump escapes	0.21	−0.02	−0.11	0.14	0.04	0.14	0.12	−0.11	−0.24	−0.00	0.24	0.10	−0.21	−0.00	−0.39	0.14	*

Values greater than 0.24 and lower than −0.24 are significant, $p < 0.05$.

TABLE 3

RANK ORDER EIGENVALUES

Factors	Eigenvalues	% Total Variance	Cumulative Eigenvalues	Cumulative %
1	2.75	16.16	2.75	16.16
2	2.43	14.29	5.18	30.45
3	2.08	12.21	7.25	42.66
4	1.81	10.68	9.07	53.34
5	1.64	9.62	10.70	62.96
6	1.13	6.63	11.83	69.59
7	1.08	6.38	12.91	75.97
8	0.80	4.70	13.71	80.66
9	0.73	4.30	14.44	84.96
10	0.65	3.81	15.09	88.77
11	0.50	2.95	15.59	91.72
12	0.39	2.29	15.98	94.01
13	0.28	1.67	16.27	95.68
14	0.26	1.51	16.52	97.20
15	0.20	1.16	16.72	98.36
16	0.16	0.95	16.88	99.31
17	0.12	0.69	17.00	100.00

approaching rat, mice showed active flight behavior, and when they ran to escape the chasing rat, they frequently displayed RA consisting of an abrupt movement arrest often followed by orientation to the oncoming threat stimulus. Sometimes, a reversal of movement to approach the rat was observed during the chase. Furthermore, when mice were constrained in one part of the runway, periods of active RA, consisting of approaches to the rat, followed by withdrawals, alternating with immobility phases were observed. Finally, defensive threat and attack to the rat occurred upon forced contact.

The present analysis indicated that the behavioral measures taken in the MDTB are generally poorly related. Even responses displayed in the same phases did not always correlate with each other. It is noteworthy, for instance, that defensive vocalization did not correlate with the defensive attack reactions (i.e., biting and jump attack) recorded in the same test. This suggests that defensive vocalization is unrelated to these other behaviors and can occur independently of changes in defensive attack. Despite weak correlations, the factor analysis indicated that the parameters obtained from the MDTB contributed to 5 main Factors. Three measures from the chase test (stops, orientations, and reversals) correlated with each other, and loaded negatively on Factor 1. Thus, Factor 1 appears to primarily relate to RA. The specificity of this is emphasized by the approach/withdrawal RA measure, which has an opposite (positive; although borderline) loading to that of the 3 aforementioned chase measures. RA consists of various information-gathering activities that occur primarily in the context of uncertainty concerning the threat characteristics of the stimulus (4). Because of a potential isomorphism between RA activities and certain key features of generalized anxiety disorder (e.g., hypervigilance, apprehensive expectation, and scanning), it was suggested that they may represent a pattern of responses particularly sensitive to anxiolytic drug challenge (4). This idea was subsequently confirmed by extensive pharmacological investigations showing that traditional (BZ) as well as novel (5-HT-related compounds) anxiolytics selectively decreased these responses (7). Thus, parameters with high loadings on Factor 1 can reasonably be interpreted as measures of anxiety, although it is not clear at this stage why jump attacks should load (positively), albeit less heavily, on this Factor.

Approach/withdrawal behaviors recorded in the straight alley test, although corresponding to RA activities, loaded heavily on a different Factor than the RA responses recorded during the chase test, thereby suggesting that they relate to a different aspect of anxiety including approach-avoid conflict. Unlike approach/withdrawal, which corresponds to active RA, orientations and stops in the chase/flight test mainly consist of RA responses seen during movement arrest. Interestingly, recent findings from the MDTB showed that BZ receptor ligands (alprazolam, chlordiazepoxide, Ro 19-8022, and Ro 19-4603) exclusively affected RA in the chase test, and 5-HT_{1A} receptor ligands (8-OH-DPAT and gepirone) specifically modulated RA

TABLE 4

ORTHOGONAL FACTOR LOADINGS FROM THE MOUSE DEFENSE TEST BATTERY

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Avoidance distance	–	–	−0.51	–	–
Avoidance frequency	–	–	−0.52	0.45	–
Flight speed	–	–	−0.55	–	–
Reversals	−0.73	–	–	–	–
Head orientations	−0.91	–	–	–	–
Stops	−0.78	–	–	–	–
Approaches/withdrawals	0.41	−0.76	–	–	–
Closest distance between animals	–	0.68	−0.45	–	–
Immobility/freezing	–	–	−0.57	–	−0.43
Vocalizations	–	–	–	–	–
Upright postures	–	–	–	0.65	–
Bitings	–	–	−0.46	0.44	–
Jump attacks	0.46	–	–	–	–
Line crossings	–	−0.64	–	–	–
Rearings	–	–	–	–	0.78
Wall climbings	–	–	–	–	−0.58
Jump escapes	–	–	–	0.66	–

Loadings < 0.40 have not been included.

elicited in the straight alley situation (11–13). Therefore, it seems that Factor 1 relates to a form of anxiety that is particularly sensitive to BZ receptor ligands, and Factor 2 relates to anxiety involving primarily 5-HT neurotransmission through 5-HT_{1A} receptors. This view is further supported by the observation that 5-HT_{1A} receptor agonists significantly reduced line crossings that negatively loaded on Factor 2.

The 3 flight measures (avoidance distance and frequency and flight speed), together with 2 responses from the straight alley test (closest distance between animals and freezing/immobility) and bitings toward the rat correlated with each other, and highly loaded on Factor 3. Thus, an array of nonRA active defenses have a negative loading on this Factor. The only apparent exception is immobility. However, it is noteworthy that immobility in the mouse is not like in the rat, a prevalent response mode that interferes with active behaviors. Instead, in the mouse, it may be compatible with other active defenses, and indeed may be a high tonus preparation for action because it is very brief and is typically interspersed with active responses. Like RA, these responses all have relatively straighforward parallels in human behavior, being seen clearly, for example, in a situation involving conspecific attack (5). Furthermore, previous studies with the MDTB demonstrated that flight behaviors may be of particular interest in the modelling of panic-like reactions as panicolytic drug treatment specifically decreased these responses (10,12). These studies also revealed that the 2 panicolytic agents imipramine and fluoxetine markedly reduced immobility/freezing time when the mouse was trapped in a straight alley. Finally, they demonstrated that compounds effective against generalized anxiety and panic attacks significantly attenuated the frequency of biting the rat and potential anxiogenic drug treatment (the BZ inverse agonist Ro 19-4603) potentiated this response. It is therefore likely that Factor 3, like Factors 1 and 2, relates to anxiety. However, unlike the latter, Factor 3 reflects a more "affective"-orientated defense (5). Similarly, parameters with high loading on Factor 4 also involved very intense defense reactions that corresponded either to flight/escape behaviors (avoidance frequency and postrat jump escapes) or to defensive threat/attack responses (upright posture and biting). These are obviously active nonRA defenses, but it is not clear how they differ from the active nonRA defenses of Factor 3 (except that Factor 3 has negative loadings and this one has positive loadings). They may represent terminal defenses. An alternative is to regard them as defenses to specific threat stimuli, in that upright, biting, and avoidance are defenses to a specific stimulus, but jump-escape treats the situation as a specific stimulus.

Finally, variables (immobility/freezing, postrat wall rearing, and climbings) that showed moderate to high loadings on Factor 5 have in common that they all occurred in situations where there was no oncoming/approaching rat or direct contact with the threatening stimulus, either because the rat remained at constant distance (straight alley) or because it has been removed from the area (posttest). Because rearing is the item that loaded most heavily on this Factor, and this behavior was seen during contextual defense testing, one can consider that Factor 5 reflects escape from an area in which danger has been recently encountered. The finding of an opposite correlation between wall climbings and immobility/freezing on one hand, and wall rearings on the other hand, obviously reflects a shift in intensity of response from the more intense response (i.e., wall climbing and freezing/immobility) to a less intense one (i.e., wall rearing), and thus suggest that Factor 5 relates to anxiety. If we take the view, fairly well substantiated in rats, that immobility is a situational defense, then the negative loading for immobility is compatible with the view that this Factor represents reduced situational defensiveness. However, on the basis of our recent drug findings, it is difficult to understand the meaning of this Factor, because it was not significantly affected by any specific anxiogenic (BZ inverse agonist) or anxiolytic (BZ agonists, 5-$HT_{1A/2A}$ ligands, 5-HT reuptake inhibitors) drug treatment, nor by very high and motor-impairing doses of the compounds used in these studies, thereby excluding the possibility that Factor 5 is a reliable index of motor activity (10–13). A remaining possibility is that it relates to behavioral processes that do not primarily involve GABA/BZ system or 5-HT neurotransmission. Clearly, further investigations with other pharmacological agents than those used in these studies are warranted for a better understanding of the meaning of Factor 5.

In conclusion, the results of the present factor analysis suggest that the behaviors scored in the MDTB may relate to different aspects of anxiety. Although Factor 1 and Factor 2 manifestly include cognitive aspects of defensive behavior that appear to be related to the process of acquiring and analyzing information in the present of threatening stimuli, Factor 3 and Factor 4 reflect more "affective"-orientated defense reactions. These findings thus confirm that the MDTB, in addition to serving as an excellent procedure for investigation of neurobehavioral systems, provides an animal model for several human emotional states.

REFERENCES

1. Belzung, C.; Le Pape, G. Comparison of different behavioral test situations used in psychopharmacology for measurement of anxiety. Physiol. Behav. 56:623–628; 1994.
2. Beuzen, A.; Belzung, C. Link between emotional memory and anxiety states: A study by principal component analysis. Physiol. Behav. 58:111–118; 1995.
3. Blanchard D. C.; Blanchard R. J. Ethoexperimental approaches to the biology of emotion. In: Rosenzweig M. R.; Porter L. W., eds. Annual review of psychology. Annual Reviews Inc.: Palo Alto, CA; 1988:43–68.
4. Blanchard D. C.; Blanchard R. J.; Rodgers R. J. Risk assessment and animal models of anxiety. In: Olivier B.; Mos J.; Slangen J. L., eds. Animal models in psychopharmacology. Birkhauser Verlag A. G.: Basel; 1991:117–134.
5. Blanchard R. J.; Blanchard D. C. Affect and aggression: an animal model applied to human behavior. In: Blanchard R. J.; Blanchard D. C., eds. Advances in the study of aggression. Academic Press: Orlando, FL; 1984:1–62.
6. Blanchard, R. J.; Flannelly, K. J.; Blanchard, D. C. Defensive behavior of laboratory and wild *Rattus norvegicus*. J. Comp. Psychol. 100:101–107; 1986.
7. Blanchard, R. J.; Yudko, E. B.; Rodgers, R. J.; Blanchard, D. C. Defense system psychopharmacology: An ethological approach to the pharmacology of fear and anxiety. Behav. Brain Res. 58:155–165; 1993.
8. Cruz, A. P. M.; Frei, F.; Graeff, F. G. Ethopharmacological analysis of rat behavior on the elevated plus-maze. Pharmacol. Biochem. Behav. 49:171–176; 1994.
9. File, S. E. Behavioural detection of anxiolytic action. In: Elliott J. M.; Heal D. J.; Marsden C. A., eds. Experimental approaches to anxiety and depression. John Wiley & Sons Ltd: Chichester; 1992:25–44.
10. Griebel, G.; Blanchard, D. C.; Agnes, R. S.; Blanchard, R. J. Differential modulation of antipredator defensive behavior in Swiss-Webster mice following acute or chronic administration of imipramine and fluoxetine. Psychopharmacology 120:57–66; 1995.

11. Griebel, G.; Blanchard, D. C.; Jung, A.; Blanchard, R. J. A model of 'antipredator' defense in Swiss-Webster mice: Effects of benzodiazepine receptor ligands with different intrinsic activities. Behav. Pharmacol. 6:732–745; 1995.
12. Griebel, G.; Blanchard, D. C.; Jung, A.; Lee, J. C.; Masuda, C. K.; Blanchard, R. J. 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; 1995.
13. Griebel, G.; Blanchard, D. C.; Jung, A.; Masuda, C. K.; Blanchard, R. J. 5-HT_{1A} agonists modulate mouse antipredator defensive behavior differently from the 5-HT_{2A} antagonist pirenperone. Pharmacol. Biochem. Behav. 51:235–244; 1995.
14. Lister, R. G. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 92:180–185; 1987.
15. Rodgers R. J. Effects of benzodiazepine and 5-HT receptor ligands on aggression and defense in animals. In: Rodgers, R.J.; Cooper, S. J., eds. 5-HT_{1A} agonists, 5-HT_3 antagonists and benzodiazepines. their comparative behavioural pharmacology. John Wiley & Sons: Chichester; 1991:195–231.
16. Rodgers, R. J.; Johnson, N. J. T. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacol. Biochem. Behav. 52:297–303; 1995.