Chapter 6

The Mouse Defense Test Battery: A Model Measuring Different Facets of Anxiety-Related Behaviors

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Abstract

Defensive behaviors of lower mammals constitute a significant model for understanding human emotional disorders. They generally occur in response to a number of threatening stimuli, including predators, attacking conspecifics, and dangerous objects or situations. Such behaviors can readily be studied in wild rats, wild mice, or in several laboratory mice, which show a complete defensive repertoire in response to danger. Here we describe the mouse defense test battery (MDTB), which measures flight, freezing, defensive threat and attack, and risk assessment in response to an unconditioned predator stimulus, and postthreat (conditioned) defensiveness to the test context. The MDTB represents a significant improvement over other animal models for evaluating drugs active against emotional disorders since it is capable of responding to and differentiating anxiolytic drugs of different classes through specific profiles of effect on different measures.

Key words: Mouse defense test battery, Anxiety, Panic, Defense repertoire, Risk assessment, Flight, Defensive aggression

1. Background and Historical Overview

Defensive behaviors occur in response to a number of threatening stimuli, including predators, attacking conspecifics, and dangerous objects or situations. Such behaviors can readily be studied in wild rats which show a complete defensive repertoire in response to danger. In contrast, in laboratory rats, defensive threat and attack behaviors in response to predators have been much reduced through systematic selection for docility by breeders (1). However, the disadvantages of using wild rats as subjects in laboratory research are obvious. For example, it is clear that the difficulty and cost in obtaining and maintaining these animals are greater than for laboratory rats.

Todd D. Gould (ed.), *Mood and Anxiety Related Phenotypes in Mice: Characterization Using Behavioral Tests*, *Volume II*, Neuromethods, vol. 63, DOI 10.1007/978-1-61779-313-4_6, © Springer Science+Business Media, LLC 2011

There are reasons to believe that the laboratory mouse has not been so severely selected on the basis of its defensive behaviors. The smaller size of the mouse and its reduced potential to inflict serious wounds, plus the ease of handling mice with a tail pickup, have enabled greater tolerance of defensive attack behavior in this species and, indeed, domesticated mice often show biting behavior to human handling (2). Thus, it has been demonstrated that mice from four lines, three inbred (BALB/c, C57BL/6, and DBA/2) and one outbred (Swiss), show intense defense reactions when confronted with an approaching threat stimulus (laboratory rat). They display initial flight, followed by risk assessment (RA) and defensive vocalization, and biting occur when escape is blocked (3). The concept of RA has emerged from the work of Blanchard and colleagues (4). These authors defined RA in terms of orientation toward present or potential threat, often followed by specific approach responses. They demonstrated that RA is associated with gathering of information concerning threat sources. Together, these defense patterns closely resemble those of wild rats, suggesting that mice of these strains do not show the reductions in flight and defensive threat/attack that are typical of laboratory rats. Such findings clearly indicate that the laboratory mouse may be a suitable subject for studies concerned with defensive behaviors.

However, it was not clear in these initial studies whether the responses displayed by the mice were specific to the encounter with a laboratory rat. The idea that defensive reactions may be elicited by any approaching stimulus was addressed by studying the influence of various stimuli on defensive reactions of Swiss mice (5). Briefly, this study demonstrated that when compared to mice approached by a leather glove, animals confronted with an anesthetized or a conscious rat displayed potentiated flight responses and defensive threat/attack reactions, while RA behavior was generally similar in all three conditions. Furthermore, escape attempts following removal of the stimulus were higher in the rat conditions compared to the leather glove group. In this latter case, however, responses displayed by the leather glove group mice were also higher than those observed in a group which was not exposed to any stimulus, indicating that the leather glove stimulation also elicited defense reactions, albeit at a lower level. Taken together, these results demonstrated that a rat stimulus elicits higher levels of flight reactions and defensive threat/attack responses than a leather glove stimulus, thereby suggesting that this experimental situation is appropriate for investigating antipredator defense.

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 MDTB 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 (6). 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 MDTB (see below). Results suggest that certain defensive behaviors may be considered particularly relevant in modeling 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 modeling 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 MDTB and several behaviors often described in generalized anxiety disorder patients (7) such as apprehensive expectation, and vigilance and scanning, involving hyperattentiveness (8). In addition, the observation that panic disorder patients usually report an urgent desire to flee from where the attack is occurring (8) has led to suggest that panic symptoms are due to pathological, spontaneous activation of neuronal mechanisms underlying flight reactions (9, 10). As such, flight behaviors in the mouse defense test battery may model certain aspects of panic (11).

2. Equipment, Materials, and Setup

2.1. Animals

The Swiss strain of mouse is recommended for use in this protocol, because of the animals' high levels of defensiveness (see also point 4 below). Mice are 10–12-week-old at the beginning of the experiment. The stimulus subject is a Long Evans male rats (400–500 g). The use of another strain of rat as a stimulus has not been studied extensively. Some preliminary findings tend to suggest that the Long Evans strain is the most suitable as it elicits more stronger defensive behaviors in mice than Wistar or Sprague–Dawley rats.

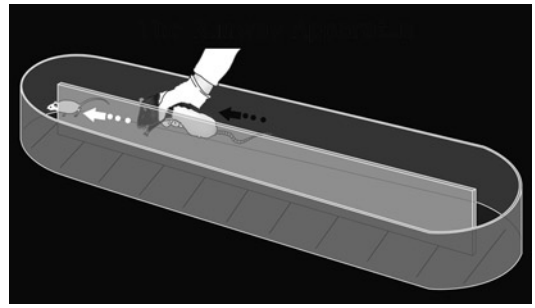

Fig. 1. Runway apparatus.

Both mice and rats are housed singly in polycarbonate cages in a room maintained under a 12-h light/dark cycle with light onset at 6 a.m. Although it is possible to use a live rat as stimulus, it is highly recommended, for obvious ethical reasons, to use an anesthetized (e.g., with 40 mL/kg of pentobarbital) or a freshly terminated (killed by CO_2 inhalation) rat.

2.2. Test Environment

Experiments are performed in a quiet, darkened test room away from disturbance and under red light to minimize visual contact with the experimenter.

2.3. Setup

Two video cameras are mounted vertically above the runway apparatus and connected to a television screen located in an adjacent room. They allow the live recording of pre- and posttest activities. Assessments of defensive responses are made from the recordings with the observer unaware of the original pretreatment.

2.4. Apparatus

The test is conducted in an oval runway, 0.40 m wide, 0.30 m high, and 6.0 m in total length, consisting of two 2 m straight segments joined by two 0.4 m curved segments and separated by a median wall ($2.0 \times 0.30 \times 0.06$) (Fig. 1). The apparatus is 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 are made of black Plexiglas. The floor is marked every 20 cm to facilitate distance measurement. Experiments are performed under red light between 1 p.m. and 5 p.m.

3. Procedure

3.1. Subjects and Drug Administration

Mice are brought into a holding area immediately adjacent to the test room at least 1 h before testing. Animals must be randomly allocated to the various drug groups and injected at the interval appropriate to the route of injection of a particular drug.

3.2. Familiarize Test Subjects to the Test Arena: The Pretest (Minutes 1–3)

Place a mouse in the middle of the runway apparatus. Allow 3 min of free exploration and count line crossings, wall rears, wall climbs, and jump escapes (Fig. 2a).

3.3. Rat Avoidance Test (Minutes 4–6)

Immediately after the 3-min familiarization period, introduce the hand-held stimulus rat at one end of the runway, 2 m from the subject. Bring it up to the subject at a speed of approximately 0.5 m/s, initiating approach only if the subject is at a standstill with its head oriented towards the hand-held rat. Consequently, intervals between trials are variable but never exceeded 15 s. Terminate approach when contact with the subject is made or the subject runs away from the approaching rat. If the subject flees, record avoidance distance (the distance from the rat to the subject at the point of flight). Remove the rat from the apparatus between each trial so that there is no visual contact between the threat stimulus and the subject. Repeat for a total of five approaches (Fig. 2b).

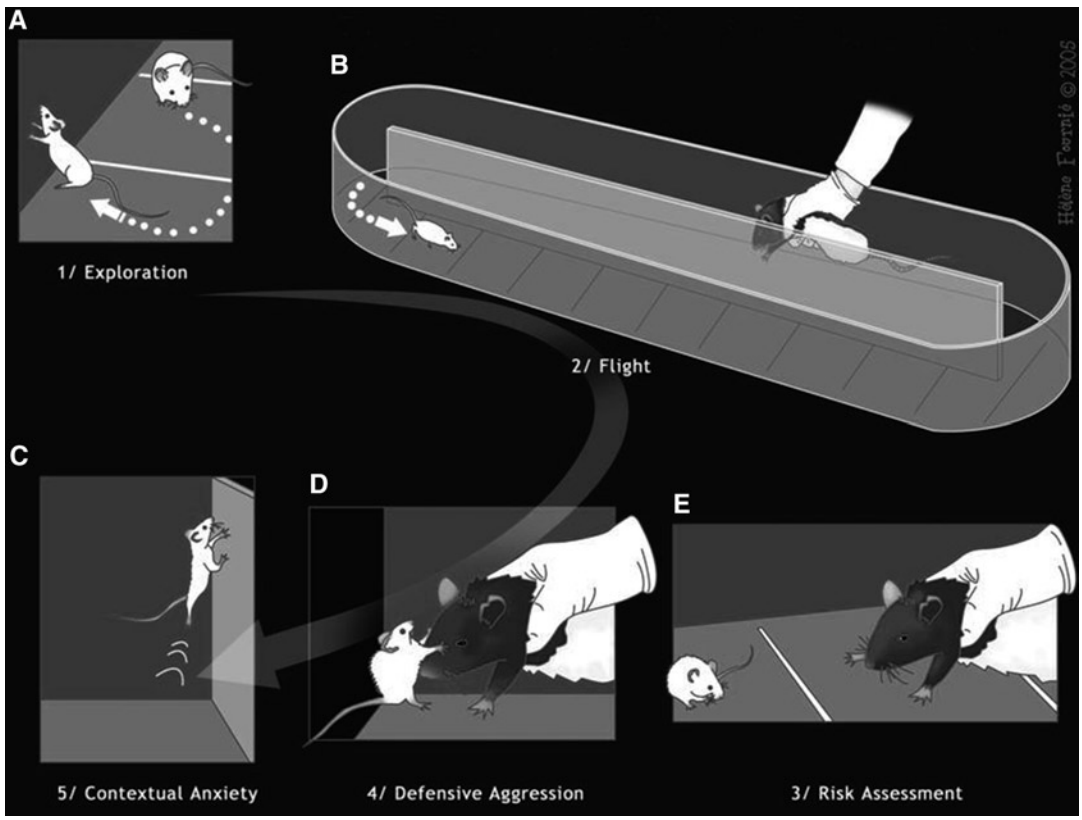

Fig. 2. The different behaviors displayed by mice in the runway apparatus before (exploration), during (flight, risk assessment, and defensive aggression), and after (escape attempts elicited by contextual anxiety) exposure to a Long Evans rat.

3.4. Chase/Flight Test (Minutes 7–8)

Introduce hand-held rat at a distance of 2 m from the subject and initiate chase only when subject is at a standstill with its head oriented toward the rat. Bring rat up to the subject at a speed of approximately 2 m/s. Chase is terminated when the subject has traveled a distance of 15 m. During chase, maintain a constant distance of 20 cm between the two animals. Consequently, if subject stops fleeing before traveling the full 15 m, stop the chase too in order to avoid contact between the two animals; resume by moving the hand-held rat quickly from left to right in front of the subject to elicit flight. Record the following parameters: 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). Remove the rat after the chase is completed (Fig. 2e).

3.5. Straight Alley Test (Minutes 9–11)

By the closing of two doors (60 cm distant from each other), the runway is then converted to a straight alley in which the subject was constrained. The rat is introduced in one end of the straight alley. Session is initiated when (1) the subject faces the rat; (2) both animals are 40 cm distant from each other. During 30 s, following measures are taken: immobility time, closest distance between the subject and the rat, and the number of approaches/withdrawals (subject must move more than 20 cm forward from the closed door, then return to it). The hand-held rat remains at the place it is introduced during the full 30 s. After this session, it is removed from the straight alley area.

3.6. Forced Contact Test (Minutes 12–13)

Bring the rat up to contact the subject in the straight alley. Direct approaches quickly (within 1 s) to the subject's head. For each such contact, note bites, vocalizations, upright postures, and jump attacks by the subjects. Remove the rat from the apparatus if no defensive threat and/or attack responses are elicited within 15 s. Repeat this test three times. The time interval between each trial is approximately 5 ± 1 s (Fig. 2d).

3.7. Posttest (Minutes 14–16)

Remove the rat immediately after the forced contact test and open the doors to convert straight alley back to an oval runway. Record line crossings, wall rears, wall climbs, and jump escapes (Fig. 2c) during a 3-min session.

3.8. End of Testing

After removal of each animal, the runway field must be carefully mopped using hot soapy water to remove any residual odor due to urine, faces, or to the rat stimulus.

4. Data Analysis and Anticipated Results

Data are analyzed by a one-way analysis of variance (ANOVA) (avoidance distance, flight speeds, immobility time, and closest distance between animals) or the nonparametric Kruskal–Wallis test for some infrequently occurring or highly variable behaviors (avoidance frequencies, reversals, head orientations, approaches/withdrawals, bites, vocalizations, upright postures, and jump attacks). Subsequent comparisons between treatment groups and control is carried out using Newman–Keuls procedures or the nonparametric Mann–Whitney *U* test. Pre- vs. posttest differences are evaluated by a combined repeated measures ANOVA followed by a Newman–Keuls posthoc comparison (line crossings) or by the Mann–Whitney *U* test and Wilcoxon matched pair test if the behavior occurs infrequently (wall climbings and jump escapes).

Strain differences are particularly pronounced in the MDTB. When subjects are chased by the rat, C57BL/6 mice use flight as the dominant defense strategy, while the defensive responses of BALB/c, C57BL/6, and DBA/2 mice consist of flight reactions and RA activities. However, when flight or escape is not possible, RA becomes the predominant feature of the defense repertoire in the C57BL/6 mice. When defensive threat/attack behaviors are required, Swiss, BALB/c, DBA/2, and C57BL/6 mice show very similar reactions in terms of the magnitude of the responses observed. CBA mice are poorly defensive in all these test situations. Finally, after the rat is removed from the test apparatus, Swiss, DBA/2, and C57BL/6 mice generally display more vertical activities than BALB/c mice (12). Although in a few experiments female mice have been used, little has been published by way of validation and the influence of the estrus cycle has not been investigated.

Anxiolytic compounds should decrease defensive behaviors, whereas anxiogenic drugs should show opposite effects. However, some responses may be specifically or mainly affected by certain drug classes. Figure 3 shows a graphical representation on how the different classes of drugs affect defensive responses elicited in the MDTB. For example, benzodiazepines (BZs) decrease RA activities of animals chased by the rat and defensive threat and attack responses, while 5-HT_{1A} agents mainly affect contextual defense and defensive threat and attack behaviors. In addition, high-potency BZs, such as alprazolam and clonazepam, as well as antidepressants belonging to the tricyclics, monoamine-oxydase inhibitors, and selective 5-HT reuptake inhibitors have a clearer impact on flight responses than on other defensive reactions. Moreover, drugs that act on the hypothalamic–pituitary–adrenocortical (HPA) axis, including CRF_1 and V_{1b} receptor antagonists, have been shown to

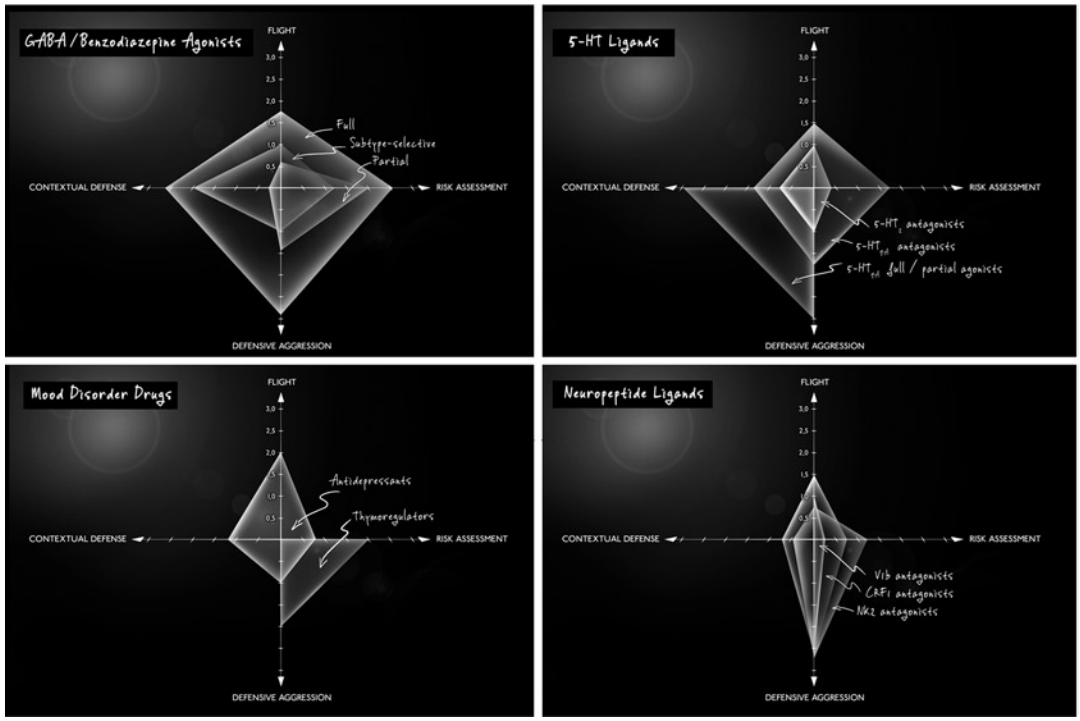

Fig. 3. Graphical representation on the efficacies of a variety of psychoactive compounds on the different emotional behaviors in the mouse defense test battery: flight, risk assessment, defensive aggression, contextual defense. Scores range from 0 (no efficacy) to 3 (highly effective in reducing defensive responses). For more details, see (13).

attenuate the defensive aggression parameters, while leaving the other behaviors unchanged (for a review, see (13)). Taken together, these observations suggest that RA, flight, defensive threat/attack, and escape attempts probably reflect different aspects of anxiety-related reactions, thereby confirming the findings from the factor analysis.

5. Experimental Variables and Troubleshooting

5.1. Training

The MDTB requires about 1 week training for the pre- and post-test measures to be scored reliably, but longer for the defensive measures. A skilled experimenter may be able to perform the test in a satisfactory manner after 2 weeks of practice. A well-trained experimenter is necessary, who is capable to quickly evaluate multiple defensive behaviors, some of which may not be obvious at first glance. Scoring can be live or from tape and must be performed by an observer blind to the drug treatment and test condition.

5.2. Inadequate Levels of Defensive Behaviors

The main problem seems to be the use of batches of animals or strains that give rise to low levels of defensiveness. If this occurs, increase the period of individual housing from 7 to 14 days. Low levels of defensiveness may also occur if the experimental room temperature exceeds 24°C.

5.3. Sedative Drug Effects

The pretest horizontal and vertical activities provide information on sedative or stimulant effects of a drug. These measures can be used to determine the specificity of any changes in defensiveness. If a compound has marked sedative effects, it is likely that all aspects of defensive behaviors will be reduced.

5.4. Reuse of Animals

Use animals only once. Repeated testing may change the nature of the anxiety and hence will also change pharmacological responses. Generally, reexposure to the runway apparatus and the stimulus rat tends to reduce defensiveness.

5.5. Fatigue of the Experimenter

Performing the MDTB requires to be in good shape since the experimenter moves constantly around the runway apparatus and from the holding area to the experimental room. To avoid fatigue and decrease in concentration, limit the testing to about 12–16 animals per day, depending on the experimenter.

6. Concluding Remarks

The MDTB was developed from tests of defensive behaviors in rats, reflecting earlier studies of responses of laboratory and wild rodents to threatening stimuli and situations. It was designed to examine anxiogenic- or anxiolytic-like properties of psychoactive drugs through effects on specific defensive behaviors. Principal component analysis has suggested that the behaviors scored in this procedure may relate to different aspects of anxiety, which relate either to the process of acquiring and analyzing information in the presence of threatening stimuli or to more affective-orientated defense reactions. The MDTB represents a significant improvement over other animal models for evaluating drugs active against emotional disorders since it is capable of responding to and differentiating anxiolytic drugs of different classes through specific profiles of effect on different measures.

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