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Genetic Differences in the Mouse Defense Test Battery

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The mouse defense test battery (MDTB) has been designed to examine anxiogenic- or anxiolytic-like properties of psychoactive drugs through effects on specific defensive behaviors. In the present study, the MDTB was used to evaluate the potential contribution of genetic factors to these behaviors. The data revealed pronounced differences in several defense reactions among four inbred strains (BALB/c, C57BL/6, CBA, DBA/2) and one outbred (Swiss) mouse line. Thus, when subjects were introduced into the apparatus, Swiss and C57BL/6 displayed the highest levels of horizontal and vertical activities, while BALB/c and DBA/2 mice showed intermediate and CBA low activity rates. When subjects were chased by the rat, C57BL/6 mice used flight as the dominant defense strategy, while the defensive responses of BALB/c, C57BL/6, and DBA/2 mice consisted of flight reactions and risk assessment activities. However, when flight or escape was not possible, risk assessment became the predominant feature of the defense repertoire in the C57BL/6 mice. When defensive threat/attack behaviors were required, Swiss, BALB/c, DBA/2, and C57BL/6 mice showed very similar reactions in terms of the magnitude of the responses observed. CBA mice were poorly defensive in all these test situations. Finally, after the rat was removed from the test apparatus, Swiss, DBA/2, and C57BL/6 mice displayed more vertical activities than BALB/c mice. These latter, however, showed an increased level of ambulation compared to the activity recorded before the rat exposure. Together, these findings indicate that genetic factors contribute to defensive behaviors in this animal model of anxiety. The different behavioral profiles displayed by the strains used here may provide the means to obtain a better insight into the neurobehavioral mechanisms involved in anxiety-related disorders. Aggr. Behav. 23:19–31, 1997. © 1997 Wiley-Liss, Inc.

Key words: **defensive behaviors; flight; risk assessment; defensive threat/attack; anxiety; strain comparison; mice**

INTRODUCTION

Defensive behaviors occur in response to a number of types of threatening stimuli, including predators, attacking conspecifics, and dangerous objects or situations. The suggestion has been made many times that the defensive responses constitute a signifi-

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cant model for understanding human emotional disorders [e.g., Blanchard and Blanchard, 1984]. Studies using a visible burrow system have been used to characterize the overall pattern of defensive behaviors of rats and mice to a potential threat (e.g., a cat) [Blanchard and Blanchard, 1989; Blanchard et al., 1995b]. These analyses were followed by the creation of test batteries in which particular defensive behaviors can be individually elicited and their response to drugs determined. The fear/defense test battery (F/DTB) measures rat reactions (flight, freezing, defensive threat, and defensive attack) to an approaching and contacting predator, while the anxiety/defense test battery (A/DTB) primarily measures risk assessment (RA) and inhibition of nondefensive behavior to potential threat. The more recent mouse defense test battery (MDTB) combines many of the features of the F/DTB and the A/DTB into a single procedure, eliciting and measuring reactions to both present and anticipated threats. In a mouse-scaled oval runway, mice show an extremely precise delineation of defensive behaviors including flight, avoidance, sonic vocalization, and defensive threat/attack, with each behavior controlled by specifiable characteristics of the threat stimulus and situation, just as in rats. The major mouse difference from rats is in RA. As an example, during flight, mice stop periodically, look back at the approaching rat, and sometimes change their flight direction. The MDTB has proven useful for evaluating potential anxiolytics [Griebel et al., 1995a–d]. Moreover, it may be used to differentiate the effects of drugs effective against generalized anxiety (GAD) as opposed to panic disorders (PD), through effects on specific behaviors. For instance, tests with panicolytics (i.e., alprazolam, imipramine, and fluoxetine) indicated that these reduced flight reactions [Griebel et al., 1995a,b] were not altered by drugs used in the clinical management of GAD (e.g., chlordiazepoxide, gepirone) [Griebel et al., 1995b,d]. These latter drugs mainly reduced RA responses, activities which were little affected by panicolytic compounds. This pharmacological specificity led to the proposal that the MDTB may represent an effective animal model of GAD and PD [Griebel et al., 1995c].

Many authors have provided evidence that fear/anxiety-motivated behaviors vary among stocks and strains. For instance, several studies using the open field revealed differences in the reactivity to a new environment between various strains of mice [Streng, 1971; Archer, 1977; Peeler and Nowakowsky, 1987; Crusio et al., 1991; Makino et al., 1991]. As an illustration, Makino et al. [1991] demonstrated that BALB/c mice showed strong and long-lasting stretching immediately after their introduction into the field, while C57BL/6 and DBA/2 mice never displayed such behavior. Instead, they immediately started to move around. These authors interpreted their findings in terms of "emotional arousal," with the BALBc strain being more "anxious" than the two other lines. More recently, Beuzen and Belzung [1995], using several tasks based on exploratory behavior (e.g., the light/dark choice test and the free exploration procedure), confirmed that BALBc generally show a more pronounced reluctance to locomote in a novel area than do C57BL/6 and DBA/2 mice.

These differences in the behavioral repertoire of mice of different genotypes are likely to carry over into other situations to which these animals have not yet been exposed. Thus, the aim of the present study was to investigate further the contribution of genetic heterogeneity on fear-motivated behavior. To this end, mice from four inbred strains (BALBc, C57BL/6, CBA, and DBA/2) and from one outbred line (Swiss) were tested in the MDTB. Swiss mice were used because they are among the most aggressive laboratory strains with reference to both offensive and defensive forms of intraspecific attack [Parmigiani et al., 1989]. The inbred lines were chosen on the basis of differences in behavioral profile as revealed in several models of anxiety [Roullet and Lassalle, 1990; Makino et al., 1991; Griebel et al., 1993; Beuzen and Belzung, 1995].

MATERIALS AND METHODS

Animals

Subjects were 50 naive male mice from four inbred strains (BALB/cByJIco, C57BL/6JIco, CBA/JIco, and DBA/2JIco) and from one outbred line (Swiss) aged 9 weeks at the time of testing, and 6 male Long Evans rats (400–500 g). They were obtained from Iffa-Credo (L'Arbresle, France). Prior to experimental testing, they were housed singly to 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 hr light/dark cycle with light onset at 6 a.m.

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 two 2 m straight segments joined by two 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. In addition, the apparatus was equipped with infrared beams and sensors capable of measuring the velocity of the animal during the chase/flight test. Experiments were performed under red light between 9 a.m. and 2 p.m.

Procedure

Pretest: 3-min familiarization period. Subjects were placed into the runway for a 3-min familiarization period, in which line crossings, wall rears, wall climbs, and jump escapes were recorded (min 1–3).

Reactions to the Rat

Rat avoidance test (min 4–6). Immediately after the 3-min familiarization period, a hand-held dead rat (killed by CO_2 inhalation) was introduced five times at one end of the runway and brought up to the subject (at a speed of approximately 0.5 m/sec) which was at the other end of the apparatus so that they were separated by a constant distance of 2 m at the initiation. Approach was initiated only if the subject was at a standstill with its head oriented toward the hand-held rat. Consequently, intervals between trials were variable but never exceeded 15 sec. 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. The rat was removed from the apparatus between each trial so that there was no visual contact between the predatory stimulus and the subject.

Chase/flight test (min 7–8). The hand-held rat was brought up to the subject at a speed of approximately 2.0 m/sec. As was the case in the rat avoidance test, a constant distance of 2 m separated the rat and the subject when the former was introduced in the runway. Chase was initiated only when the subject was at a standstill with its head oriented toward the hand-held rat. Chase was completed when the subject had traveled a distance of 15 m. During the chase, a constant distance of 20 cm was maintained between the two animals. Consequently, if the animal stopped fleeing before traveling the full 15 m, the chase was stopped too in order to avoid contact between the two animals. The experimenter then moved the hand-held rat quickly from left to right in front of the subject to elicit flight. 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). The rat was removed after the chase was completed.

Straight alley (min 9–11). By the closing of two doors (60 cm distant from each other), the runway was then converted to a straight alley in which the subject was constrained. The rat was introduced in one end of the straight alley. Session was initiated when 1) the subject faced the rat; 2) both animals were 40 cm distant from each other. During 30 sec, the following measures were 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 remained at the place it was introduced during the full 30 sec. After this session, it was removed from the straight alley area.

Forced contact (min 12–13). Finally, the experimenter brought the rat up to contact the subject in the straight alley. Approaches were directed quickly (within 1 sec) to the subject's head. For each such contact, bites, vocalizations, upright postures, and jump attacks by the subjects were noted. If no defensive threat and/or attack responses were elicited within 15 sec, the rat was removed from the apparatus. This was repeated three times. The time interval between each trial was approximately 5 ± 1 sec.

Post test: Contextual Defense

Immediately after the forced contact 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 14–16).

Statistical Analysis

Data were 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 ANOVA 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 were carried out using Newman-Keuls procedures or the nonparametric Mann-Whitney U-test. Pre- vs. posttest differences were evaluated by a combined repeated-measures ANOVA followed by a Newman-Keuls post-hoc comparison (line crossings) or by the Mann-Whitney U-test and Wilcoxon matched pair test if the behavior occurred infrequently (wall climbings and jump escapes). In addition, the unbiased correlation ration or eta squared (η^2) [Winer, 1971; Reuchlin, 1976; Hedges and Olkin, 1985] was calculated from the ANOVA table for each dependent variable to obtain a measure of the magnitude of the effect and an estimate of the proportion of the variance attributable to genetic factors. Nonparametric data are displayed as mean ± standard error in order to illustrate the group variation.

RESULTS

Behaviors Before and After Rat Exposure (Table I)

Pretest: 3-min familiarization period. Significant between-strain effects were observed for line crossings [$F(4,45) = 12.7, P < 0.001$] and wall rearings [$F(4,45) = 7.5, P < 0.001$], but not for wall climbings [$H(4,50) = 6.23$] and jump escapes [$H(4,50) = 4$]. The highest η^2 was 0.49 for line crossings, for wall rearings the η^2 was 0.35. These η^2 values indicate that 49 and 35% of the variance in line crossing and wall rearing, respectively, could be explained by genetic factors. Newman-Keuls analysis revealed that these mouse strains could be divided into three distinct groups with respect to line crossings and two groups with respect to wall rearings. Thus, CBA displayed the lowest ambulation responses while the other strains clustered in a high activity range in which several strains (Swiss and C57BL/6) showed the highest ambulation scores. Regarding wall rearings, CBA and BALB/c displayed the lowest activity.

Posttest: contextual defense. After the removal of the rat, significant between-strain differences in the runway activities were observed for line crossings [$F(4,45) = 8.4, P < 0.001$], wall rearings [$F(4,45) = 13.1, P < 0.001$], wall climbings [Kruskal-Wallis: $H(4,50) = 25.08$, $P < 0.001$], but not for jump escapes [Kruskal-Wallis: $H(4,50) = 3.88$]. The magnitude of η^2 was 0.38, 0.50, and 0.37 for line crossings, wall rearings, and wall climbings, respectively. As is shown in Table I, BALB/c mice displayed the highest number of line crossings, while CBA showed the lowest. For the other strains, activity was distributed in a continuum between the intermediate-low activity of DBA/2 mice and the intermediate-high activity of C57BL/6 and Swiss mice. Wall-rearing activities were comparable for DBA/2, Swiss, and C57BL/6 mice and significantly greater than those obtained in BALB/c and CBA mice. Finally, Swiss mice displayed more wall climbings than the other strains. The average scores of these latter did not differ from each other.

Pre- vs. posttest comparison. ANOVA indicated that there were significant strain × test interactions for line crossings [$F(4,45) = 8.72, P < 0.001$], wall rearings [$F(4,45) = 2.56$, $P < 0.05$], wall climbings [Friedman: $N(1,50) = 31.11$, $P < 0.001$], and jump escapes [Friedman: $N(1,50) = 6$, $P < 0.05$]. Post-hoc analyses revealed that line crossings significantly increased in the posttest in BALB/c and CBA mice, while a similar effect was observed on wall rearings and climbings in Swiss, BALB/c, DBA/2, and C57BL/6 mice. Finally, the analysis showed that DBA/2 mice displayed significantly more jump escapes in the posttest than during the initial period.

Reactions to the Rat

Rat avoidance test. Data are presented in Table II. A significant effect of strain was observed for both measures: avoidance distance: $F(4,41) = 4.6, P < 0.01$ and avoidance frequency: $H(4,50) = 23.28, P < 0.001$. The η^2 values for these analyses were 0.24 and 0.40, respectively. CBA displayed the lowest avoidance distance scores, while C57BL/6 showed the highest. With respect to avoidance frequency, the rank order of the strains was different from that obtained for the avoidance distance. Thus, DBA/2 mice displayed the highest frequency, BALB/c, Swiss, and C57BL/6 mice intermediate frequencies, and CBA mice the lowest score for this parameter.

Chase/flight test. ANOVA revealed a significant strain effect on speed: $F(4,42) = 5.1, P < 0.01$; reversals: $H(4,47) = 12.45, P < 0.05$; and stops: $F(4,42) = 4.3, P < 0.01$,

TABLE I. Behaviors Displayed by Several Strains of Mice Before (Pre) and After (Post) Rat Exposure in the MDTB†

	Swiss	BALB/c	CBA	DBA/2	C57BL/6
Pre line crossings	$110.9 \pm 10.71^{B,C,D}$	$81.1 \pm 12.30^{S,C,c5}$	$36.5 \pm 5.21^{S,B,D,c5}$	$80.5 \pm 7.04^{S,B,c5}$	$108.8 \pm 3.79^{B,C,D}$
Pre wall rearings	$5.8 \pm 1.67^{B,C}$	$1.3 \pm 0.54^{S,C,c5}$	$0.5 \pm 0.22^{S,D,c5}$	$6 \pm 1.37^{B,C}$	$8.6 \pm 1.67^{B,C}$
Pre wall climbings	0.3 ± 0.15	0 ± 0	0 ± 0	0.1 ± 0.1	0.7 ± 0.60
Pre jump escapes	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.5 ± 0.5
Post line crossings	$109.3 \pm 19.32^{B,C}$	$138.8 \pm 7.94^{S,C,D,c5,*}$	$69.1 \pm 8.32^{S,B,*}$	79.3 ± 4^{B}	$91.0 \pm 11.3^{B,C}$
Post wall rearings	$17.2 \pm 2.13^{B,C,*}$	$10.8 \pm 1.89^{S,C,D,c5,*}$	$5.0 \pm 1.01^{S,B,D,c5}$	$19.0 \pm 4.15^{B,C,*}$	$16.9 \pm 1.26^{B,C,*}$
Post wall climbings	$9.5 \pm 2.27^{B,C,D,c5,*}$	$2.5 \pm 0.85^{S,*}$	0.1 ± 0.10^{S}	$3.1 \pm 0.69^{S,*}$	$4.0 \pm 1.01^{S,*}$
Post jump escapes	1.9 ± 0.90	0.8 ± 0.33	0.7 ± 0.70	$3.8 \pm 3.04^{*}$	2.7 ± 1.81

†s, significantly different from Swiss; B, significantly different from BALB/c; C, Significantly different from CBA; D, significantly different from DBA/2, c5, significantly different from C57BL/6.
*Significantly different from pretest ($P < 0.05$).
Data represent M ± SEM.

TABLE 2. Avoidance Responses Displayed by Mice From Several Strains Facing an Approaching Rat*

	Swiss	BALB/c	CBA	DBA/2	C57BL/6
Avoidance distance (cm)	85.3 ± 19.32^{C}	$63.4 \pm 7.94^{C,c5}$	$35.8 \pm 8.32^{S,B,D,c5}$	$78.4 \pm 4.00^{C,c5}$	$101.5 \pm 11.3^{B,C,D}$
Avoidance frequency	3.4 ± 0.54^{C}	$4.0 \pm 0.37^{C,c5}$	$1.5 \pm 0.40^{S,B,D}$	$4.6 \pm 0.03^{C,c5}$	$2.3 \pm 0.45^{B,D}$

*s, significantly different from Swiss; B, significantly different from BALB/c; C, significantly different from CBA; D, significantly different from DBA/2, c5, significantly different from C57BL/6. Data represent M ± SEM.

but not on head orientations: $H(4,47) = 8.36$. η^2 values for the significant parameters were 0.26, 0.16, and 0.23, respectively. Table III shows that two different groups were observed with regard to flight speed and reversals. Thus, C57BL/6 showed the highest speed velocity and the lowest reversal scores, while all other strains displayed comparable performances on both measures. With respect to stops, Swiss mice displayed the highest tendency to stop, while the other strains showed comparable activities.

Straight alley. Significant between-strain effects in the approach/withdrawal [$H(4,50) = 18.68$, $P < 0.001$] and the closest distance between animals [$F(4,45) = 5$, $P < 0.01$] measures were observed. The magnitude η^2 was 0.26 and 0.25, respectively. There were no significant strain differences for immobility time [$F(4,45) = 0.5$]. Mann-Whitney analysis revealed two distinct groups for the frequency of approaches/withdrawals. Table IV shows that when facing a rat which remained at a constant distance, DBA/2 and CBA mice displayed a low level of approach/withdrawal activity (i.e., 0.7 and 0.8, respectively), while average scores for all other strains ranged from 2.2 to 2.6. With respect to the closest distance between animal measure, DBA/2 stayed significantly farther from the rat than the other strains. These can be further divided into intermediate (CBA and BALB/c) and short (Swiss and C57BL/6) distance groups.

Forced contact. When subjects were confronted by the rat, Table V shows that significant between-strain differences were observed for vocalizations [$H(4,50) = 14.36$, $P < 0.001$], defensive upright postures [$H(4,50) = 25.89$, $P < 0.001$], bitings [$H(4,50) = 22.22$, $P < 0.001$], but not for the frequency of jump attacks [$H(4,50) = 3.85$]. The magnitude of η^2 was 0.31, 0.60, and 0.48 for vocalizations, uprights, and bitings, respectively. Regarding these latter measures, Newman-Keuls analyses revealed that while Swiss, BALB/c, DBA, and C57BL/6 mice displayed comparable scores, they all showed a higher level of defensive reactions than CBA mice.

DISCUSSION

The present results provide further evidence that mice exhibit intense defense reactions in response to rat stimuli in the oval runway cage. Thus, in response to an approaching rat, subjects showed active flight behavior, and when they ran to escape the chasing rat, they frequently showed RA consisting of an abrupt movement arrest often followed by orientation to the oncoming rat. 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.

Although Swiss, BALB/c, CBA, DBA/2, and C57BL/6 mice showed a rather consistent pattern of defensiveness across tests, the data demonstrated that behaviors exhibited in the MDTB varied with genotype, depending on what defense strategy was required.

Runway Activities Before and After Rat Exposure

When subjects were introduced in the runway cage, their tendency to explore a novel environment was more or less pronounced, depending on the strain. Thus, Swiss and C57BL/6 displayed the highest levels of horizontal and vertical activities, while BALB/c and DBA/2 mice showed intermediate and CBA low activity rates. These results are akin to previous findings showing that Swiss and C57BL/6 mice have a natural ten-

TABLE 3. Behaviors Exhibited by Mice From Several Strains Chased by a Rat in the Oval Runway Cage*

	Swiss	BALB/c	CBA	DBA/2	C57BL/6
Flight speed (m/sec)	0.73 ± 0.11	0.48 ± 0.04^{c5}	0.45 ± 0.04^{c5}	0.54 ± 0.03^{c5}	1.00 ± 0.17^{B,C,D}
Reversals	1.9 ± 0.50^{c5}	1.5 ± 0.60^{c5}	1.7 ± 0.36	2.3 ± 0.50^{c5}	0.2 ± 0.13^{S,B,D}
Head orientations	4.2 ± 1.05	2.3 ± 0.60	1.6 ± 0.37	1.4 ± 0.52	1.3 ± 0.40
Stops	6.1 ± 1.13^{C,c5}	3.7 ± 0.96	2.4 ± 0.48^{S}	3.9 ± 0.69	1.6 ± 0.52^{S}

*Significantly different from Swiss; B, significantly different from BALB/c; C, significantly different from CBA; D, significantly different from DBA/2, c5, significantly different from C57BL/6. Data represent M ± SEM.

TABLE 4. Behaviors Exhibited by Mice From Several Strains in the Straight Alley Test*

	Swiss	BALB/c	CBA	DBA/2	C57BL/6
Approaches/withdrawals	2.6 ± 0.48^{C,D}	2.2 ± 0.55^{C,D}	0.8 ± 0.20^{S,B,c5}	2.2 ± 0.25^{S,B,c5}	2.2 ± 0.25^{C,D}
CDBA (cm)	5.9 ± 4.03^{D}	10.9 ± 5.09^{D}	18.0 ± 4.95	29.6 ± 5.68^{S,B,c5}	5.1 ± 2.05^{D}
Immobility (s)	6.9 ± 2.49	7.5 ± 1.21	5.9 ± 0.98	5.2 ± 1.23	4.7 ± 1.64

*CDBA, closest distance between animals; S, significantly different from Swiss; B, significantly different from BALB/c; C, significantly different from CBA; D, significantly different from DBA/2; c5, significantly different from C57BL/6. Data represents M ± SEM.

TABLE 5. Behaviors Exhibited by Mice From Several Strains Upon Forced Contact With a Rat*

	Swiss	BALB/c	CBA	DBA/2	C57BL/6
Vocalizations	2.5 ± 0.27^{c}	3.0 ± 0.00^{c}	$1.4 \pm 0.43^{s,B,D,c5}$	3.0 ± 0.00^{c}	2.4 ± 0.27^{c}
Upright postures	2.6 ± 0.31^{c}	2.9 ± 0.10^{c}	$0.3 \pm 0.30^{s,B,D,c5}$	2.9 ± 0.10^{c}	2.2 ± 0.33^{c}
Bitings	0.6 ± 0.34^{c}	2.8 ± 0.13^{c}	0.7 ± 0.33	0.9 ± 0.28	0.6 ± 0.31
Jump attacks	0.6 ± 0.31	0.2 ± 0.13	0.7 ± 0.33	0.9 ± 0.28	0.6 ± 0.31

*Significantly different from Swiss; B, significantly different fro mBALB/c; C, significantly different from CBA; D, significantly different from DBA/2; C5, significantly different from C57BL/6. Data represent M ± SEM.

dency to actively explore unknown areas, while BALB/c and especially CBA mice were strongly inhibited [Griebel et al., 1990, 1993; Beuzen and Belzung, 1995]. For instance, when given the choice between a familiar area and a novel one, Swiss and C57BL/6 mice more actively explored the unknown area than the two other strains. C57BL/6 mice have been found to be active locomotors and BALB/c inactive in earlier studies using the open field [Fredericson, 1953; Thompson, 1953; McClearn, 1959; Van der Pool and Davis, 1962; De Fries and Hegmann, 1970; Rose and Parsons, 1970; Streng, 1971; Makino et al., 1991]. These findings led to the suggestion that BALB/c can be described as "emotional" mice and C57BL/6 as "nonemotional" [Robertson, 1979].

Previous findings from the MDTB have suggested that subjects' activities after the removal of the rat can be considered as intense escape attempts in a potential threatening situation [Griebel et al., 1995a–d]. The present data are in line with these findings as they revealed an increase in vertical activities (e.g., wall rearings) compared to similar responses recorded during the pretest, regardless of strain. Nevertheless, strain differences were apparent. Thus, Swiss, DBA/2, and C57BL/6 mice displayed the highest level of vertical escape attempts. It must be emphasized, however, that these increases were proportionally bigger with BALB/c and CBA mice than with the three other strains. For instance, wall rearings increased by 700–900% in the case of the two former, while the average percentage increases for Swiss, DBA/2, and C57BL/6 mice were 196, 217, and 96, respectively. It is, however, difficult to suggest that rat exposure less profoundly impacted vertical activities of these three strains in comparison to BALB/c and CBA mice. Indeed, in the case of the former, pre/posttest comparisons were made from a much higher pretest level, so that their vertical activities could hardly be increased by 700–900% as was the case in BALB/c and CBA mice. Regarding line crossings, only BALB/c and CBA mice showed significant increases in this measure in the posttest. This effect, at least in BALB/c mice, cannot be explained by a lower pretest level of line crossings as DBA/2 mice, which showed quite comparable performances during the initial period than BALB/c, which did not display an increase in horizontal motor activities in the posttest. Clearly, rat exposure more severely affected ambulation of BALB/c and CBA mice than the other strains.

Runway Activities During Rat Exposure

In the rat avoidance test, a situation which permits the subject to run away and out of sight of the rat, flight was the dominant response for Swiss, BALB/c, and DBA/2 mice as revealed by the frequency of avoidance scores (68, 80, and 92%, respectively). CBA were poorly responsive as only 30% of the rat's approaches elicited flight. Interestingly, although C57BL/6 mice showed an intermediate score in the number of trials on which avoidance occurred (48%), when they did flee their avoidance distance was reliably superior to that of BALB/c, CBA, DBA/2 and, to a lesser extent, of Swiss mice. This indicates that when flight was the strategy chosen by C57BL/6 mice, it occurred rapidly after the introduction of the rat. Otherwise, contact was made between the subject and the threatening stimulus. The lower avoidance distance for Swiss, BALB/c, and DBA/2 mice indicates that the latency to flee was increased, thereby suggesting that they spent more time assessing a potential danger (i.e., an approaching rat) than did C57BL/6 mice. Alternatively, it may be argued that differences in avoidance distance simply reflect differences in visual acuity or attentiveness rather than defensiveness per se. However, the observation that Swiss, BALB/c, and DBA/2 mice displayed more RA responses than did C57BL/6 mice would suggest that the avoidance distance performances mainly reflect defensiveness. In the chase/flight test, C57BL/6 mice chose flight as the dominant defense strategy, while defensive responses of the other strains comprised flight reactions as well as RA activities. Among these latter strains, CBA mice were the least defensive during the chase. In particular, they displayed fewer stops than Swiss and, to a lesser extent, BALB/c and DBA/2 mice. The behavioral profiles observed in the chase/flight test indicates that different style of defensive responses were used by animals. C57BL/6 mice chose to flee by the quickest and most direct route, whereas Swiss, BALB/c, and DBA/2 employed protean strategies (i.e., random rapid changes in direction, frequent stops) which aimed at confusing the chasing rat [Drivers and Humphries, 1988]. These patterns of defensive behaviors may be of particular interest in view of recent drug findings indicating that flight reactions were primarily affected by panic-modulating agents (e.g., yohimbine, alprazolam, imipramine) [Blanchard et al., 1993; Griebel et al., 1995a,b], whereas RA responses (i.e., stops and orientations) were decreased by drugs known to alleviate GAD (e.g., chlordiazepoxide, gepirone) [Griebel et al., 1995b,d]. This would suggest that C57BL/6 mice are more suitable for the study of panicolytics, while Swiss, BALB/c, and DBA/2 mice appear to be very appropriate subjects for research on drugs expected to be effective in the clinical management of GAD.

In the straight alley test, strain differences were not observed for all measures. Thus, immobility time was equivalent for all strains. By contrast, active RA (i.e., the number of approaches/withdrawals) was strongly reduced in the CBA and DBA/2 mice compared to the other strains. The defense strategy used by these two latter strains is ambiguous. They did not freeze as immobility barely reached 20% of the total time for both lines, nor did they actively assess the threatening stimulus. Instead, they both remained distant from the rat. Perhaps a more detailed analysis of their behavior (e.g., vertical activities, stretch attend postures, body orientations) during this phase would establish what defense strategy (if any) was used in the straight alley. Interestingly, in this particular test situation, C57BL/6 mice displayed as many RA responses as the outbred line and BALB/c mice, indicating that when escape is not possible, RA becomes the predominant feature of the defense repertoire in the C57BL/6 mice.

The terminal components of the defense pattern, defensive threat (vocalization and upright posture), biting, and jump attacks, which occurred upon forced contact, were very similar in Swiss, BALB/c, DBA/2, and C57BL/6 mice. Particularly, subjects showed a high level of defensive threat reactions and bitings. Obviously, when neither flight nor RA is a possible strategy, these four strains exhibit the same pattern of defensiveness. These findings are somewhat at variance with those recently obtained by Blanchard et al. [1995a] in a similar situation. They showed that C57BL/6 mice displayed significantly fewer defensive threat/attack reactions than Swiss-Webster mice when subjects were confronted by an anesthetized rat. Differences in methodology may account for this apparent discrepancy. For instance, in Blanchard's study, subjects had never experienced the rat stimulus before this phase, nor were they familiar with the test area in which they encountered the rat. Finally, as was the case in the previous phases, CBA mice were set apart from all the other strains since they showed considerable reductions in the number of trials on which these defensive behaviors occurred.

In conclusion, the present findings provide evidence for an influence of genetic factors on several defensive behaviors which have been shown to be specifically sensitive to clinically effective anxiolytic and panic-reducing drugs [Griebel et al., 1995a–d]. The observation that C57BL/6 mice primarily use flight as a defense strategy in the chase/flight test may be of particular interest for the study of the neural mechanisms underlying PD as panic-modulating drugs specifically modulate this behavior. The finding that three of the four strains studied, Swiss, BALB/c, and DBA/2 mice, used RA as the dominant feature of the defense strategy, and that RA was also the dominant defensive behavior for C57BL/6 mice when escape was not possible, adds to the view that RA is a major component of defensive behavior in mice as well as in rats, and strengthens analyses [Blanchard et al., 1991] suggesting that homologous neurobehavioral RA systems in people may be involved in the etiology and manifestations of anxiety and anxiety disorders.

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