

## Behavioral profile of wild mice in the elevated plus-maze test for anxiety

A. Holmes<sup>a,\*</sup>, S. Parmigiani<sup>b</sup>, P.F. Ferrari<sup>c</sup>, P. Palanza<sup>b</sup>, R.J. Rodgers<sup>d</sup>

<sup>a</sup>Section on Behavioral Neuropharmacology, Experimental Therapeutics Branch, National Institute of Mental Health, Building 10, Room 4D11, Bethesda, MD 20892-1375, USA

<sup>b</sup>Dipartimento di Biologia Evolutiva e Funzionale, Università di Parma, Viale di Scienze, Parma 43100, Italy

<sup>c</sup>Istituto di Fisiologia Umana, Università di Parma, via Volturno 39, Parma 43100, Italy

<sup>d</sup>Ethopharmacology Laboratory, School of Psychology, University of Leeds, Leeds LS2 9JT, UK

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### Abstract

Systematic observations of the defensive behavior of wild rodents have greatly informed the experimental study of anxiety and its neural substrates in laboratory animals. However, as the former work has been almost exclusively carried out in rats, few data are available concerning the reactivity of wild mice to standardized tests of anxiety-related behavior. In the present experiments, we employed ethological measures to examine the behavioral responses of a wild-derived population of house mice (*Mus musculus*) in the elevated plus-maze. In direct comparisons with laboratory Swiss mice, male wild mice exhibited substantially elevated levels of exploratory activities and an overall “preference” for the open arms of the plus-maze. On re-exposure to the plus-maze, male wild mice showed further increases in open arm exploration, while Swiss mice showed a marked shift to the enclosed parts of the plus-maze. Tested over a single session, female wild mice also exhibited a profile of high open arm exploration, but showed levels of exploratory behaviors and locomotor activity similar to female Swiss counterparts. While exploratory patterns in wild mice show similarities to profiles seen in certain laboratory strains (e.g., BALB/c), wild mice displayed a number of additional behaviors that are unprecedented in plus-maze studies with laboratory mice. These included actual and attempted jumps from the maze, spontaneous freezing, and exploration of the upper ledges of the closed arms. Thus, while in conventional terms the behavior of wild mice was consistent with one of low anxiety-like behavior, the presence of these unique elements instead indicates a profile more accurately characterized by high reactivity and escape motivation. We discuss how the use of an ethological approach to measuring plus-maze behavior can support accurate interpretation of other exceptional profiles in this test, such as those possibly arising from phenotyping of transgenic and gene knockout mice. © 2000 Elsevier Science Inc. All rights reserved.

**Keywords:** Wild mice; Elevated plus-maze; Anxiety; Escape; Ethological methods

### 1. Introduction

Over the past 15 years, the elevated plus-maze test has become the most widely used animal model for the study of drug effects on anxiety [1,2] and, more recently, has found widespread application in the behavioral phenotyping of transgenic and knockout mice (e.g., Refs. [3–12]). In the conventional form of the test, anxiety is routinely assessed by measures of open arm avoidance while locomotor activity is most reliably measured by the frequency of closed arm entries [2]. However, despite the general utility

of this two-dimensional perspective, detailed video analysis has identified a number of additional behavioral acts and postures characteristic of intact mice [13] and rats [14] exposed to the maze. These ethological elements, which include stretched attend postures (SAP), head-dipping, and grooming, have been linked through factor analysis to risk assessment, directed exploration, and displacement activity, respectively [15–18]. Furthermore, recent pharmacological studies have shown that the incorporation of such measures in plus-maze scoring not only reduces the likelihood of false positives and negatives [13,14,19,20], but also enhances the sensitivity of the model to novel anxiolytics (e.g., Refs. [21–24]).

Much of the impetus underlying the application of ethological techniques to the elevated plus-maze came from studies on rodent defensive behavior and, in particular, the

\* Corresponding author. Tel.: +1-301-496-4838; fax: +1-301-480-1164.

E-mail address: aholmes@codon.nih.gov (A. Holmes).

elegant work of the Blanchards on antipredator defense in wild rats. These studies demonstrated that in response to the approaching predatory threat of a cat or human, wild rats exhibit a range of context-specific defenses including flight/escape, freezing, and defensive threat/attack (e.g., Refs. [25,26]). Consistent with the impact of domestication, comparative studies revealed that laboratory rats exhibit a considerable attenuation of defensiveness (e.g., Refs. [26,27]). Parallel findings have more recently been reported for mice. Thus, despite remarkable similarities in social, aggressive, and predatory behavior (e.g., Refs. [28–31]), antipredator flight responses in Swiss CD-1 laboratory mice are significantly less intense than those exhibited by the wild house mouse (*Mus musculus*) [32].

To date, however, there has been little research on the behavior of wild rodents in commonly used laboratory models of anxiety, such as the elevated plus-maze. In one study [33] of the defensive reactivity of wild mice (*M. musculus*) to predator-related stimuli, the ‘explosive’ response of wild mice to the test apparatus per se totally precluded assessment of their behavior. Furthermore, although wild voles (*Microtus socialis*) show patterns of elevated plus-maze exploration similar to those seen in laboratory rodents (i.e., open arm avoidance), wild spiny mice (*Acomys dimidiatus*) display an apparent “preference” for the open arms [34]. In the present study, novel methodology was employed to directly compare plus-maze behavior of laboratory mice (Swiss CD-1) with the responses of the progenitor of all laboratory strains, the wild house mouse (*M. musculus*). In Experiment 1, the behavior patterns of wild and laboratory males were contrasted over two consecutive daily plus-maze trials and, in Experiment 2, a comparison was made between wild and laboratory females on a single plus-maze trial.

## 2. Methods

### 2.1. Animals

For Experiment 1, subjects were 11 male Swiss CD-1 laboratory mice (Charles River, Italy) and 14 male mice from a wild-derived population. For Experiment 2, subjects were 20 virgin female Swiss CD-1 laboratory mice (Charles River, Italy) and 17 virgin female mice from a wild-derived population. All subjects were approximately 13 weeks of age at the time of testing. Wild-derived mice were laboratory-bred for four generations from a founder population of house mice (*M. musculus*) originally trapped in Grosseto, Tuscany, Italy. In line with previous studies with laboratory-bred wild mice, the description of wild-derived mice will be abbreviated to “wild mice” (e.g., Ref. [35]). At weaning (30 days), wild mice were housed with two to three same-sex littermates ( $40 \times 25 \times 20$  cm<sup>3</sup>). Swiss CD-1 mice were housed in groups of 10/cage ( $40 \times 25 \times 20$  cm<sup>3</sup>). Wild and Swiss CD-1 mice were maintained in separate, temperature-

( $21 \pm 1^\circ\text{C}$ ) and humidity- ( $50 \pm 5\%$ ) controlled vivaria, under a 12-h reversed light cycle (lights off at 0700 h). To minimize handling on the day of testing, all mice were individually housed (cages:  $33 \times 15 \times 13$  cm<sup>3</sup>) 24 h prior to initial plus-maze exposure. Food and drinking water were freely available except during brief test periods. All animals were experimentally naive at the start of the study.

### 2.2. Apparatus

The elevated plus-maze comprised two open arms ( $30 \times 5 \times 0.25$  cm<sup>3</sup>) and two closed arms ( $30 \times 5 \times 15$  cm<sup>3</sup>) that extended from a common central platform ( $5 \times 5$  cm<sup>2</sup>). The apparatus was constructed from Plexiglas (white floor, clear walls) and elevated to a height of 60 cm above floor level. Open arm exploration was encouraged by the inclusion of a slightly raised edge (0.25 cm) around their perimeter, and by testing under dim red light ( $1 \times 100$  W, indirect) (e.g., Refs. [20,36]). All sessions were videorecorded by a camera positioned above and at approximately  $50^\circ$  to the maze.

### 2.3. Procedure

#### 2.3.1. Experiment 1

On test days, male mice were transported to the dimly illuminated laboratory and left undisturbed for at least 1 h prior to testing. As wild mice are very reactive to direct human handling, a cylindrical cardboard tube (8 cm in diameter and 35 cm in length) was used to individually transport all test animals (laboratory/Swiss and wild) from their home cages to the plus-maze. The open end of the tube was placed in the home cage, and mice gently encouraged to enter. Once inside, mice were transported a few meters to the plus-maze and introduced to the apparatus by placing the open end of the tube on the center platform (facing an open arm) and slowly tilting the tube until the animal emerged. The tube was carefully removed, and the experimenter slowly and quietly withdrew from the immediate vicinity of the maze. At the end of the 5-min test session, mice were encouraged to re-enter the tube and immediately returned to the home cage. The order of testing was counterbalanced for strain, with the maze thoroughly cleaned (wet and dry cloths) between successive sessions. Twenty-four hours after initial exposure (= Trial 1), all animals were re-exposed to the plus-maze (= Trial 2), employing an identical procedure.

#### 2.3.2. Experiment 2

With the exception that animals were exposed to the plus-maze on one occasion only, the procedure for female mice was identical to that outlined for Experiment 1.

### 2.4. Behavioral measures

Videotapes were scored blind by a highly trained observer (intrarater reliability  $\geq 0.9$ ) using ethological soft-

ware (“Hindsight” [37]). Behavioral parameters comprised both conventional spatio-temporal and more recently developed ethological measures (e.g., Refs. [13,21,38]). Conventional measures were the frequencies of total, open, and closed entries (arm entry=all four paws into an arm), percent open entries [(open/total) × 100], and percent time spent in open, closed, and central parts of the maze [e.g., (time open/session duration) × 100]. Ethological measures comprised frequency scores for supported rearing (vertical movement against the side and/or end of the walls), head-dipping (exploratory movement of head/shoulders over the side of the maze), and SAP (exploratory posture in which the body is stretched forward then retracted to the original position without any forward locomotion). In view of the importance of thigmotactic cues to rodent exploration in novel environments [39–42], head-dipping and SAPs were also differentiated as a function of their occurrence in different parts of the maze (e.g., Ref. [20]). Thus, the closed arms and center platform were designated as “protected” areas (i.e., offering relative security) and the “percent protected” scores for head-dipping and SAP calculated as the percentage of these behaviors displayed in or from the protected areas (e.g., [(protected SAP/total SAP) × 100]).

From live observations during initial testing, it was evident that wild mice displayed a number of behavioral acts and postures not previously seen in laboratory mice. To accommodate this broader behavioral repertoire, three additional measures were recorded. Percent upper ledge time was defined as the proportion of session time spent on the upper ledges of the closed arm walls [(time on ledge/300) × 100]. Jump attempts (frequency) were defined as the animal rearing on the edge of an open arm, and making movements as if preparing to jump but failing to do so.

Freezing (duration) was defined as the rapid cessation of any body movement during active exploration.

### 2.5. Statistics

Data for Experiment 1 were subjected to two-factor analysis of variance (ANOVA) (Group × Maze experience), with further comparisons performed using Newman–Keuls post-hoc tests. Measures occurring only in wild mice (i.e., percent upper ledge time, jump attempts, freezing) were analyzed by one-factor (maze experience) ANOVA. Data from Experiment 2 were analyzed by one-factor ANOVA (mouse strain).

## 3. Results

### 3.1. Experiment 1

Three male wild mice immediately jumped from the plus-maze when placed on the apparatus. As this pattern was repeated on a second attempt (see also Ref. [33]), these animals were removed from the study.

The effects of group and maze experience on plus-maze behavior in male mice are summarized in Table 1 and Fig. 1. ANOVA revealed significant Group × Maze experience interactions for percent open time, percent closed time, SAPs [all  $F_{1,19} \geq 9.26$ ,  $P < .01$ ], and percent protected SAPs [ $F_{1,17} = 278.20$ ,  $P < .001$ ]. Newman–Keuls analyses indicated that, regardless of trial, wild mice spent more time on the open arms and less time in the enclosed arms, and exhibited significantly lower levels of SAPs and percent protected SAPs. Furthermore, wild animals showed a between-trials increase in percent open time, whereas

Table 1  
Plus-maze profiles of male Swiss and male wild mice on first (Trial 1) and second (+24 h= Trial 2) exposures to the test ( $n = 10-11$ )

	Swiss		Wild	
	Trial 1	Trial 2	Trial 1	Trial 2
Total entries	20.27 ± 1.13	19.73 ± 1.78	28.90 ± 1.96***	28.20 ± 2.35***
Open entries	3.45 ± 0.78	2.73 ± 1.23	16.60 ± 2.15***	16.60 ± 2.09***
Closed entries	16.82 ± 1.02	17.00 ± 1.66	12.30 ± 1.54**	11.60 ± 0.90*
Percent open entries	16.58 ± 3.33	12.26 ± 4.98	55.92 ± 5.46***	57.54 ± 2.93***
Freezing	0 ± 0	0 ± 0	2.93 ± 2.20	7.38 ± 2.82
Supported rearing	17.18 ± 2.25	22.09 ± 4.02	23.80 ± 2.78	25.90 ± 3.90
Jump attempts	0 ± 0	0 ± 0	19.20 ± 5.32	23.50 ± 4.62
Head-dipping	6.36 ± 1.76	0.64 ± 0.28†††	11.50 ± 2.19*	10.20 ± 2.41***
Percent protected head-dipping	81.06 ± 7.40	75.00 ± 25.00	21.56 ± 7.37***	6.21 ± 2.62***
Stretched attend postures	19.18 ± 2.49	7.82 ± 1.11†††	6.40 ± 1.66***	2.90 ± 0.62*
Percent protected SAP	89.71 ± 3.13	92.81 ± 4.68	53.76 ± 10.66***	11.11 ± 6.21†††***
Grooming	5.97 ± 2.41	15.74 ± 4.76	6.99 ± 2.61	10.30 ± 2.95

Data are presented as means ± S.E.M. (see Fig. 1 for complementary data).

\*  $P < .05$  vs. Swiss/same trial.

\*\*  $P < .01$  vs. Swiss/same trial.

\*\*\*  $P < .001$  vs. Swiss/same trial.

†††  $P < .001$  vs. Trial 1/same strain.

laboratory/Swiss mice showed a significant between-trials increase in percent closed time. In addition, there was a significant intertrial reduction in SAPs in laboratory/Swiss mice, as well as an intertrial reduction in percent protected SAP in wild mice.

In addition to the above interactions, ANOVA revealed significant main effects of group for total entries, open entries, closed entries, percent open entries, percent center time, head-dipping [all  $F_{1,19} \geq 10.92$ ,  $P < .001$ ], and percent protected head-dipping [ $F_{1,11} = 33.03$ ,  $P < .001$ ]. Newman–Keuls analyses confirmed that regardless of maze experience, wild mice showed higher levels of total entries, open entries, percent open entries, and head-dipping, but lower levels of closed entries, percent center time, and percent protected head-dipping. Main effects of maze experience were found for percent center time, head-dipping, and grooming [all  $F_{1,19} \geq 5.35$ ,  $P < .05$ ]. Post-hoc analyses revealed that percent center time was significantly reduced by maze experience in both groups, while there was a between-trials reduction in head-dipping for laboratory/Swiss mice only. Percent upper ledge time, jump attempts, and freezing were identified as behaviors exhibited by wild mice only (see Table 1, Fig. 1). However, despite some trends (i.e., increases in upper ledge time and

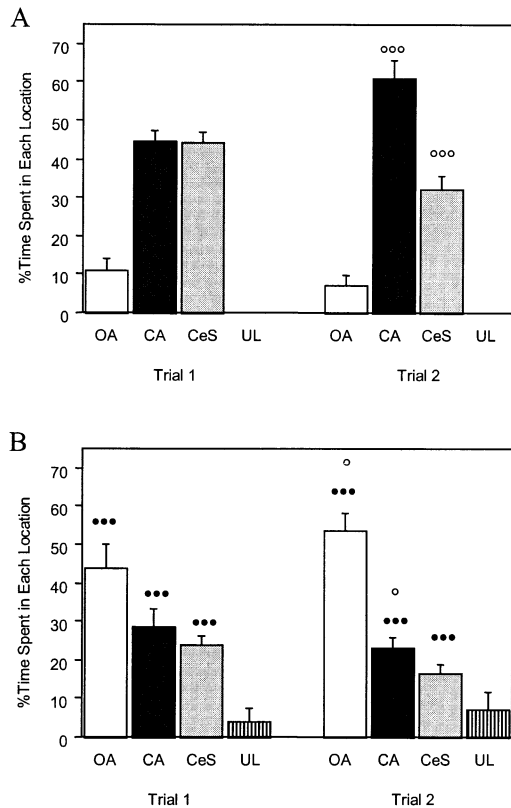


Fig. 1. Behavior of (A) male Swiss and (B) male wild mice on first (Trial 1) and second (+24 h= Trial 2) exposures to the test ( $n = 10-11$ ). Data are presented as means  $\pm$  S.E.M. \*\*\* $P < .001$  vs. Swiss/same trial; ††† $P < .001$ ; † $P < .05$  vs. Trial 1 (see Table 1 for complementary data). OA=open arms; CA=closed arms; CeS=center square; UL=upper ledge.

Table 2  
Plus-maze profiles of female Swiss and female wild mice ( $n = 17-20$ )

	Swiss	Wild
Total entries	19.50 $\pm$ 0.73	24.18 $\pm$ 3.21
Open entries	2.30 $\pm$ 0.40	11.53 $\pm$ 1.92***
Closed entries	17.20 $\pm$ 0.78	12.65 $\pm$ 1.59**
Percent open entries	11.85 $\pm$ 1.99	42.89 $\pm$ 3.80***
Freezing	0 $\pm$ 0	18.15 $\pm$ 5.34
Jump attempts	0 $\pm$ 0	7.65 $\pm$ 2.17
Supported rearing	17.60 $\pm$ 1.44	21.76 $\pm$ 3.11
Head-dipping	5.85 $\pm$ 0.77	7.18 $\pm$ 1.16
Percent protected head-dipping	74.04 $\pm$ 5.76	40.47 $\pm$ 8.11***
Stretched attend postures	11.95 $\pm$ 1.22	3.06 $\pm$ 0.52***
Percent protected SAP	94.04 $\pm$ 2.51	64.89 $\pm$ 8.48***
Grooming	1.39 $\pm$ 0.96	12.60 $\pm$ 6.46

Data are presented as means  $\pm$  S.E.M. (see Fig. 2 for complementary data).

\*\* $P < .01$  vs. Swiss.

\*\*\* $P < .001$  vs. Swiss.

freezing), none of these behaviors altered significantly across trials.

### 3.2. Experiment 2

The effects of group on plus-maze patterns in female mice are summarized in Table 2 and Fig. 2. ANOVA revealed significant effects of group for open entries, closed entries, percent open entries, percent open time, percent center time, percent protected head-dipping, SAPs, and percent protected SAPs [ $F_{1,19} \geq 7.21$ ,  $P < .01$ ]. Newman–

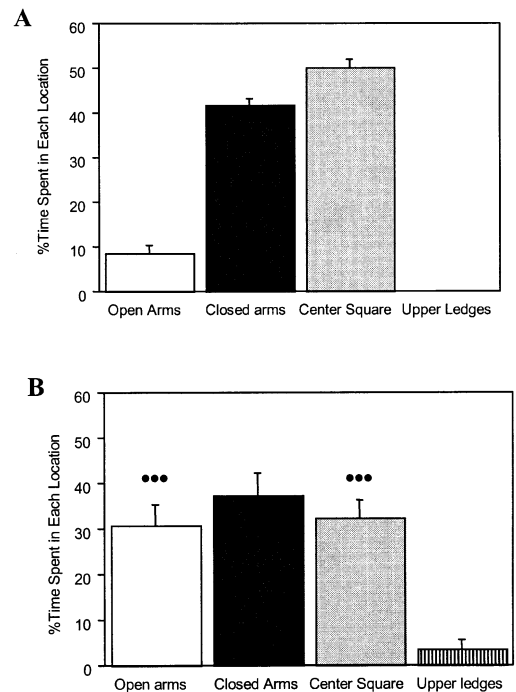


Fig. 2. Behavior of female (A) Swiss and (B) wild mice in the elevated plus-maze ( $n = 17-20$ ). Data are presented as means  $\pm$  S.E.M. \*\*\* $P < .001$  vs. Swiss (see Table 2 for complementary data).

Keuls analyses indicated that wild females displayed higher levels of open entries, percent open entries, and percent open time, but lower levels of closed entries, percent center time, percent protected head-dipping, SAPs, and percent protected SAPs. Similar to the results of Experiment 1, wild females displayed several behaviors not seen in laboratory/Swiss females. Like their male counterparts, a small amount of time was spent exploring the upper ledges of the plus-maze (i.e., ~3% of test session). However, it is noteworthy that, compared with male conspecifics, wild females tended to make fewer jump attempts and to display higher levels of freezing (Table 2 and Fig. 2).

#### 4. Discussion

To our knowledge, the current report represents the first direct comparison of the behavior of wild house mice (*M. musculus*) and laboratory mice in the elevated plus-maze, a well-validated animal model of anxiety. The results of Experiment 1 confirmed that on first exposure to the plus-maze, male laboratory/Swiss mice exhibit a clear preference for the closed/protected parts of the apparatus, a pattern previously seen both in this strain (e.g., Ref. [43]) and the closely related Swiss–Webster laboratory strain (e.g., Ref. [18]). Also as previously reported, these animals displayed a range of other responses associated with the behavioral dimensions of locomotor activity (i.e., closed arm entries, supported rearing; e.g., Ref. [18]), directed exploration (head-dipping; e.g., Refs. [20,44]), and risk assessment (SAPs; e.g., Refs. [45–48]).

In direct contrast to this profile, but similar to patterns observed in wild spiny mice [34], test-naive male wild mice showed a profound preference for the open arms (percent open time, open entries, and percent open entries). Reciprocally, these animals spent less time in “protected” parts of the maze (i.e., closed arms and center platform) and made fewer entries into the enclosed arms. Nonetheless, as a result of the very large number of open arm entries, the total arm entry score for wild males was significantly greater than that for laboratory/Swiss males. The conclusion that wild mice display higher overall activity levels in the maze is supported by the observation that despite the reduced opportunity afforded to these animals (i.e., reduced time spent in enclosed arms), laboratory/Swiss and wild mice exhibited equivalent levels of supported rearing. Furthermore, wild mice not only displayed more directed exploration (head-dipping) than their laboratory/Swiss counterparts, but the pattern of this exploration also differed between the groups. Consistent with their high levels of open arm activity, wild mice predominantly displayed head-dipping from the open arms, whereas laboratory/Swiss mice largely limited such behavior to the protected areas of the maze. Although SAPs were also biased more toward the open arms in wild mice (% SAP), overall levels of risk assessment (total SAP) were significantly lower in wild mice.

A growing number of studies have reported that prior exposure to the plus-maze can radically alter baseline behavior and/or drug responsivity on re-exposure to this test (e.g., Refs. [17,18,20,42,44,49–52]). These effects of prior maze experience have variously been attributed to sensitization [42], habituation [53], and/or a qualitative shift in the emotional response to the test [18,54,55]. Current findings extend these observations in an intriguing manner. Thus, when re-exposed to the plus-maze 24 h after Trial 1, male laboratory/Swiss mice spent significantly more time in the closed parts of the maze, and performed fewer exploratory head dips and less risk assessment behavior. Although the previously reported between-trials reduction in percent open time (e.g., Refs. [18,20]) was not observed in the present study, this discrepancy is most probably due to a floor effect, i.e., the low level of percent open time on Trial 1. Significantly, however, none of these particular test–retest changes was seen in male wild mice. In fact, the retest profile of wild mice was diametrically opposite to that of the laboratory/Swiss mice, and has little precedent in the literature. More specifically, on retest, wild mice spent significantly more time in the open arms (with a parallel reduction in closed arm time), displayed more risk assessment behaviors in the open arms (vs. closed arms), and showed a twofold (although statistically nonsignificant) increase in freezing. Thus, in addition to the major group differences observed in maze-naive subjects, present findings show that retest response patterns are also very different between wild and laboratory/Swiss mice.

The results of Experiment 2 show that with the exception of lower levels of risk assessment, the plus-maze profile of naive female laboratory/Swiss mice was largely similar to that seen in naive male conspecifics (Experiment 1). This finding is generally consistent with previous reports of minimal sex differences in the plus-maze (e.g., Refs. [38,56,57]). In accord with the pattern of group differences observed for males in Experiment 1, the most striking difference between laboratory/Swiss and wild mice was the substantially higher level of open arm exploration in female wild mice. Measures of percent open time, open entries, and percent open entries were all significantly higher in wild female mice relative to laboratory/Swiss females, as were the proportions of head dips and SAPs displayed in the open arms. However, whereas male wild mice displayed an unequivocal preference for the open arms over all other areas, female wild mice spent a similar amount of time in open arms, closed arms, and center square. Moreover, in contrast to the higher level of locomotor activity and directed exploration seen in male wild mice compared to male laboratory/Swiss, total arm entries and head-dipping were no different between wild and laboratory/Swiss female mice (although closed entries were again significantly lower in wild subjects). These findings indicate important distinctions in the plus-maze behavior of male and female wild mice, a salient finding in the context of sex differences in certain other tests for anxiety-like

behavior in rodents (e.g., Refs. [58,59]) and the clear female bias in presentation of anxiety disorders in the clinic (e.g., Ref. [60]). Furthermore, when contrasted with significantly fewer sex differences in Swiss mice, present data further attest to the disparity in plus-maze profiles between wild and laboratory/Swiss strains.

In an extensive comparison of inbred laboratory mouse strains, Trullas and Skolnick [61] characterized elevated plus-maze behavior on the basis of levels of open arm exploration. Thus, strains exhibiting low levels of open arm activity (e.g., A/J) were described as “high reactive”, while those showing high levels of activity in the open arms (e.g., C3H substrains, BALB/c substrains) were labeled “low reactive.” In these terms, the current profiles of wild mice (male and female) would be interpreted as one of low reactivity. However, while time spent in the open arms was indeed relatively high, levels of exploratory behaviors and general locomotor activity in male mice were also substantially higher than those currently observed in laboratory/Swiss mice or those previously seen in BALB/c mice in our laboratory [62]. As such, rather than displaying low reactivity to the plus-maze, the profile of male wild mice would be more accurately described as one of high reactivity. This interpretation is supported by the occurrence in wild mice of a number of behaviors that were completely absent in laboratory/Swiss mice, and which have never previously been reported in laboratory rodents exposed to the plus-maze.

Thus, male and female wild mice made a number of “jump attempts” from the open arms, a behavior that was very pronounced and which involved the animal standing on hindpaws on the edges of the open arms and making movements as if to jump, but then failing to do so. A very similar behavior has recently been reported in laboratory rats exposed to an unstable plus-maze apparatus [63,64] and interpreted as evidence of an intense anxiety (panic-like) state. In addition, wild mice actually explored the upper ledges of the closed arms, i.e., animals jumped onto the top of the closed arm walls and ambulated along the very narrow ledges. Although time spent on the upper ledges accounted for a relatively small proportion of the test session, it was nonetheless very striking and appeared to further demonstrate the escape motivation of these animals. Wild mice of both sexes also showed freezing in the plus-maze, a response that was noticeably higher in females than in males (i.e., 18 vs. 3 s). Consonant with this observation, Blanchard et al. [45] found that female rats show more movement inhibition than do males in response to potential threat.

Together with other measures reported in this study, these novel observations indicate that the behavior of both male and female wild mice was characterized by a high level of behavioral reactivity, directed towards rapid escape from the apparatus. The fact that three male mice did actually jump from the maze (and were excluded from the full analysis) supports this assertion. It is also relevant to note that

previous attempts to study anxiety-related behavior in wild mice were precluded by what was described as the “explosive” escape-directed response to the test situation per se [33]. It is possible that differences in visual abilities contributed to the differences between wild and laboratory/Swiss mice, with superior vision in wild mice working to guide their behavior in the plus-maze. However, studies have suggested that open arm exploration in the plus-maze is primarily guided by the use of thigmotactic cues (e.g., Refs. [41,42]) and that pigmented laboratory strains (e.g., C57BL/6, DBA/2) do not show more open arm exploration than albino strains, such as the Swiss strain (e.g., Refs. [16,62]). Notwithstanding, from the perspective of studies with transgenic and gene knockout mice, present findings help inform interpretation of ostensibly “anxiolytic-like” phenotypes observed in tests for anxiety-related behavior. Our data raise the possibility that a mutant mouse displaying a high level of open arm exploration in the plus-maze may not always indicate an attenuated level of anxiety-like behavior in these mice. That is, very high reactivity to elevated plus-maze exposure or abnormal visual abilities in a mutant mouse could manifest in high levels of open arm exploration, as currently reported for wild mice. In such cases, the incorporation of a broader, more ethological approach to measuring behavior could have the effect of reducing potential false-positive interpretations of abnormal behavioral profiles.

Blanchard et al. [32] have recently reported that wild mice (from the same stock as that used in the present study) exhibit markedly higher levels of flight behavior and escape attempts in response to an approaching rat predator than do Swiss (CD-1) mice. As an overwhelming urge to escape from an environment or situation can be an important feature of panic disorder [65], these authors have proposed that the flight/escape responses shown by mice in threatening contexts may be analogous to the behavioral symptoms of panic. Using a novel ‘Mouse Defense Test Battery’, they have reported substantive pharmacological evidence that the flight component of this model may indeed have predictive validity for panic disorder (e.g., Refs. [66–68]). The escape response shown by wild mice in the plus-maze (particularly males) may represent another approach to modeling intense anxiety states in animals using a simple and already widely used test apparatus. However, rather than indicating a high level of anxiety, the escape response in the plus-maze may simply demonstrate an ability in these mice to rapidly identify and explore the most likely route of escape (via the open arms). Moreover, since the behavioral pattern of escape responses seen in wild mice in the plus-maze can be understood as an adaptive response to a potentially dangerous environment, the issue arises as to whether the neural circuits underlying these responses in mice are the same as those mediating pathological anxiety states in humans. In this context, it would be important to assess the effects of clinically proven antipanic compounds (e.g., imipramine, SSRIs) on the behavioral profile of wild mice.

The currently observed differences in anxiety-related behaviors between wild and laboratory mice suggest that as for rats [25,26], the process of animal selection and domestication has had significant impact on behaviors relating to anxiety and defense. Previous studies (using the same stock of wild mice) have found few differences in intraspecific aggression [28,29] or predatory aggression [30] between wild mice and laboratory/Swiss mice. Thus, while certain aspects of the agonistic/defensive repertoire appear to have been well conserved during the process of domestication, others have apparently been attenuated. Such dissociation is compelling evidence for the existence of different subsystems underlying those behaviors generically termed “anxiety/emotion/defense.” Further comparative studies using wild mice would represent a powerful strategy for studying how these systems operate and inter-relate.

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