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Responses of Swiss–Webster Mice to Repeated Plus-Maze Experience: Further Evidence for a Qualitative Shift in Emotional State?

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HOLMES, A. AND R. J. RODGERS. *Responses of Swiss–Webster mice to repeated plus-maze experience: Further evidence for a qualitative shift in emotional state?* PHARMACOL BIOCHEM BEHAV **60**(2) 473–488, 1998.—Behavioral, endocrinological, and pharmacological data suggest that the emotional response of rodents to the elevated plus-maze alters as a function of prior test experience. In the present study, 74 intact male Swiss–Webster mice were exposed to the plus-maze for 5 min on each of 3 consecutive days, with all test sessions recorded on videotape. Behavior patterns for each trial were scored using ethological analysis software and the resultant database subjected to a number of statistical treatments. Analysis of full session profiles (i.e., 5 min total scores) showed that a single prior undrugged experience of the maze increases behavioral indices of anxiety and that these alterations are either maintained or further enhanced on subsequent trials. Furthermore, the behavioral profile evident by trial 3 was largely unchanged when animals were reexposed to the maze 10 days later. More detailed (i.e., min by min) examination of behavior patterns within and between trials demonstrated that unambiguous open arm avoidance is acquired by the third minute of trial 1, and that the behavioral profile evident by the end of trial 1 is (a) markedly different to that seen at the beginning of that trial, and (b) generally maintained or even accentuated on trials 2 and 3. The implied impact of prior test experience on future behavioral strategy in the maze was strongly supported by a series of factor analyses. Thus, while the factor associations of vertical activity and directed exploration remained constant across trials, trial 2 and 3 anxiety measures loaded on a separate factor to that loading trial 1 anxiety measures. A similar trial 1 vs. trials 2 and 3 dissociation was observed for measures of locomotor activity. Although the present findings are consistent with the proposal that prior test experience produces a qualitative shift in emotional response to the elevated plus-maze, the precise basis for this change as well as its full significance for our understanding of anxiety-related processes remain to be determined. © 1998 Elsevier Science Inc.

Plus-maze Anxiety Prior experience Between-trial Within-trial Factor analysis Qualitative change Mice

SINCE its introduction over a decade ago (28), the elevated plus-maze has become one of the most widely used animal models for the detection of anxiolytic-like activity (29). The test is based on the natural aversion of rodents for open spaces (52), has been validated for both rats (36) and mice (32,50), and is bidirectionally sensitive to manipulations designed to impact anxiety (40). The primary indices of plus-maze anxiety comprise spatiotemporal measures of open-arm avoidance, while locomotor activity is assessed either by the total number of arm entries or, more validly, by the number of closed-arm entries [e.g., (17,32)]. Several laboratories have recently reported enhanced test sensitivity through the additional scoring of certain behavioral acts and postures and, in particular, those related to the defensive pattern of risk assessment (1,11,26,41,49). Not only are these ethological measures often more sensitive to drug action, but they can also detect compounds (e.g., 5-HT_{1A} receptor partial agonists) that either fail to significantly influence open-arm avoidance per se or do so only at debilitating doses [for review, (38)].

A fascinating feature of the plus-maze concerns the effect of prior test experience on subsequent behavioral and pharmacological responses. Although early studies found stable test-retest profiles for rats and mice [e.g., (20,32,36)], it has been more widely reported that a single prior undrugged ex-

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perience of the maze significantly reduces open-arm activity in both species (2,13,15,24,25,31,44–48). This profile has been interpreted as reflecting an experientially induced sensitization of fear/anxiety (52), a state that appears to develop very early during trial 1. Thus, within-session time-bin analysis has shown that clear open-arm aversion is evident as early as the second minute of trial 1 and that it increases further throughout that session (45,48). The crucial importance of the initial stages of trial 1 is supported by significant between-session increases in open arm avoidance following a trial 1 duration of only 2 min (45), while additional observations have shown that this learning is independent of extra-maze cues (44).

Although the within- and between-trials alterations in plus-maze profiles might simply reflect a quantitative shift in anxiety/fear ("fear sensitization"), several lines of evidence would be consistent with an experientially induced qualitative shift in behavioral strategy/emotional state. Thus, on reexposure, animals exit more rapidly from the center platform, spend more time in closed arms and less time on the center platform/open arms, and show less exploratory head dipping. However, as total/closed-arm entries and rearing either remain stable or even increase on retest, this pattern of change cannot be simply attributed to a general behavioral suppression (15,45). Furthermore, unlike other anxiety models, the plasma corticosterone response to plus-maze exposure does not appear to habituate with repeated testing (22). It is, therefore, most intriguing that the efficacy of benzodiazepine anxiolytics (e.g., diazepam, chlordiazepoxide) is markedly reduced or even abolished by prior undrugged experience of the maze (15,16,20,21,23,24,32,46,47,52). This effect occurs with intertest intervals of 24 h to 2 weeks and, whereas it appears critically dependent upon trial 1 open-arm experience, it is independent of drug state on initial exposure and even the material from which the maze is constructed (16,19,20,32,46,47). Although it has been proposed that this loss of benzodiazepine efficacy may be an artefact related to between-trials habituation of locomotor activity (13), there is little evidence of a reduction in the principal measures of locomotion (total and/or closed-arm entries) upon retest [e.g., (15,16,44–47)]. A more plausible explanation is that prior experience alters the nature of the anxiety reaction provoked by this test; thus, not only are benzodiazepines ineffective in maze-experienced animals, they are ineffective against a behavioral baseline indicative of enhanced anxiety (46). This view has been further developed by File and Zangrossi (21), who suggest that trial 2 anxiety may be close to a phobic state against which benzodiazepines are known to be relatively ineffective [e.g., (34,51)]. In support of this hypothesis, factor analysis of the responses of male rats to repeated plus-maze testing have indicated that the major anxiety indices from trials 1 and 2 do indeed load on independent factors (15,23).

In view of the importance of these findings, not only to our understanding of the plus-maze per se but also to the evolution of animal models of anxiety, the present study was designed to further assess the nature of behavioral changes induced in male mice by prior plus-maze experience. Intact animals were exposed to the plus-maze on 3 successive days, response patterns were analyzed in detail by ethological scoring techniques, and the resultant database subjected to a range of statistical treatments. The mouse strain selected for study (Swiss–Webster) has been shown to be behaviorally more similar to feral mice than other laboratory strains (4,5,27,35), and to be highly suitable for pharmacological investigations of defensive reactivity in the plus-maze [e.g., (6,7)]. As previous work from this laboratory has predominantly involved inbred DBA/2 mice [but see also (10,39,42)]), a secondary aim was to gather further information relevant to strain differences in patterns of plus-maze exploration.

GENERAL METHOD

Animals

Subjects were 74 adult male Swiss–Webster mice (Bantin & Kingman Hull, UK), acquired at the age of 4–6 weeks and housed in groups of 10 per cage (45 × 28 × 13 cm) for at least 7 weeks prior to testing. They were maintained under a 12 D:12 L reversed light cycle (lights off: 0700 h) in a temperature (21 ± 1°C)- and humidity (52 ± 2%)-controlled environment. Food and water were freely available except during the brief test sessions. All mice were experimentally naive at the start of the study and, with the exception of routine husbandry, were not specifically handled prior to testing.

Apparatus

The elevated plus-maze was a modification of that validated for NIH Swiss mice by Lister (32), and comprised two open (30 × 5 × 0.25 cm) and two enclosed (30 × 5 × 15 cm) arms that extended from a common central platform (5 × 5 cm). A conventional configuration was employed in which like arms opposed one another across the central platform. The apparatus was constructed from black (maze floor) and clear (side and end walls of closed arms) Plexiglas, and was elevated on a wooden pedastel to a height of 60 cm above floor level. Open arm exploration was encouraged by the inclusion of a slight raised edge (0.25 cm) around their perimeter and by testing under dim red light (4 × 60 W indirect) (31,43).

Procedure

All experimentation was conducted during the midportion of the dark phase (1000–1400 h), and, to facilitate adaptation to the test environment, animals were transported the short distance from holding room to laboratory at least 1 h prior to testing. In total, 74 intact mice were tested on three successive occasions (intertrial interval = 24 h), with tail marking used to identify subjects. On each trial, animals were gently removed from their home cages and conveyed to the maze in individual holding boxes (33 × 15 × 13 cm). Testing commenced with the placement of a subject on the central platform of the maze (facing an open arm), following which the experimenter immediately withdrew to an adjacent laboratory. A standard 5-min test duration was employed and, between subjects, the maze was thoroughly cleaned using wet and dry cloths. Behavior was recorded by a video camera positioned above and at ca. 50° to the maze, with the signal relayed to a monitor and VCR situated outside the immediate test environment.

Behavioral Analysis

Videotapes were scored by a highly trained observer (intrarater reliability ⩾0.90) using an ethological analysis package ("Hindsight") developed by Dr Scott Weiss (Cerebrus Ltd, UK). Using separate location and behavior keys, this software permits the real-time scoring of videotapes by direct keyboard entry to a PC with the resultant datafiles electronically downloaded for statistical analysis. Parameters scored from videotape included the conventional spatiotemporal measures and a range of specific behavioral acts and postures [e.g., (43)].

Conventional measures were the frequency of open- and closed-arm entries (arm entry defined as all four paws into an arm; arm exit defined as two paws onto the central platform), total arm entries, and the amount of time spent by animals in open, central, and closed parts of the maze. These data were used to derive scores for % open entries [(open/total) × 100) and % time spent in different zones of the maze [(zone time/300) × 100). Ethological measures comprised the frequency of rearing (vertical movement against the side and/or end walls; note: mice only very rarely display unsupported rearing in this test), head dipping (exploratory movement of head/shoulders over the sides of the maze), stretched attend postures (SAP; exploratory posture in which the mouse stretches forward and retracts to original position without locomoting forward), and closed-arm returns (exiting a closed arm with forepaws only and returning/doubling back into the same arm), as well as the duration of more prolonged behaviors such as rearing, grooming (species-typical sequence beginning with snout, progressing to ears and ending with whole-body groom), sniffing (olfactory exploration of maze floor and walls, with occasional air sampling), and flatback approach (exploratory locomotion where the animal stretches to its full length while slowly moving forward). In view of the importance of thigmotactic cues to patterns of plus-maze exploration (52), head dipping, SAP, sniffing, and flatback approach were further differentiated as a function of where abouts on the maze they were displayed. Consistent with earlier reports [e.g., (43)], the closed arms and central platform were together designated "protected" areas (i.e., offering relative security), while the open arms were designated unprotected areas. Data for the above measures are presented both as behavior totals (e.g., SAP) and as "percent protected" scores (e.g., %pSAP; (protected SAP/total SAP) × 100).

Statistics

All statistical analyses were performed using the software package, "Statistica" (StatSoft Inc., Tulsa, OK). The effects of repeated testing ("trials") on gross plus-maze profiles were initially analyzed by one-way repeated measures analyses of variance (ANOVA), followed by Newman–Keuls comparisons. Two further statistical approaches were used to identify patterns of behavioral change within and between trials. First, the data for each 5-min trial were broken down into 1 min time bins and subjected to two-factor repeated measures ANOVA (trial × time bin), followed by Newman–Keuls comparisons. Second, the datasets for all three trials were subjected to factor analysis using a principal components solution with orthogonal rotation (varimax) of the factor matrices: this method ensures that the extracted factors are independent of one another. Factor pattern matrices were identified using a combination of the Kaiser criterion (eigenvalues $\geqslant 1$) and the Cattell Scree test (on a simple line plot, the point at which the smooth decrease in eigenvalues levels off to the right) (9,30). The loading of each behavioral item indicates how strongly it correlates with the associated factor/s (range −1.0 to +1.0) and, in accordance with previous studies [e.g., (11,17,32,33,53)], only factor loadings of >0.4 are reported. To facilitate comparisons with earlier multitrial studies on rats (15,23), three separate factor analyses were performed, i.e., conventional measures alone, conventional measures plus time center, and all measures.

TABLE 1

EFFECTS OF REPEATED EXPOSURE TO THE PLUS-MAZE ON BEHAVIORS SHOWN BY INTACT MALE SWISS WEBSTER MICE (n = 74).

	Trial 1		Trial 2		Trial 3	
	Mean	SEM	Mean	SEM	Mean	SEM
Total entries	20.72	0.84	17.47‡	0.74	16.04‡§	0.81
Open entries	6.70	0.56	4.14‡	0.45	4.27‡	0.53
Closed entries	14.01	0.55	13.34	0.50	11.77‡¶	0.60
%Open entries	30.27	1.89	20.75‡	1.85	23.45†	2.45
%Open time	15.97	1.21	8.81‡	0.93	7.90‡	0.93
%Closed time	26.77	0.85	38.34‡	1.75	46.36‡#	2.59
%Centre time	57.26	1.25	52.86*	1.58	45.73‡#	2.19
Rears	10.61	0.90	10.27	0.92	9.46	0.90
Rearing (duration)	6.69	0.71	8.40*	0.92	8.65*	1.01
Head-dips	27.53	1.17	13.11‡	1.04	13.84‡	1.24
%Protected head-dips	74.23	2.11	88.63‡	1.70	88.43‡	2.04
Stretched-attend postures	46.43	1.88	35.66‡	1.38	28.04‡#	1.05
%Protected SAPs	86.43	1.21	92.84‡	0.88	94.16‡	0.98
Closed arm returns	1.66	0.32	3.19‡	0.45	2.15¶	0.34
Sniffing (duration)	72.88	5.38	112.96‡	5.69	133.75‡#	6.04
%Protected sniffing	89.06	1.41	95.05‡	0.89	94.91‡	0.84
Flat-back approach (duration)	10.78	0.66	11.72	0.74	11.71	0.86
%Protected flat-back	41.35	3.56	44.67	4.44	46.41	4.56
Grooming (duration)	2.72	0.45	6.57*	0.90	11.97‡#	1.95

Intertrial interval = 24 h. SAP = Stretched attend postures.

Post-hoc analyses (Newman–Keuls comparisons); *$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ vs. Trial 1; §$p < 0.05$, ¶$p < 0.01$, #$p < 0.001$ Trial 3 vs. Trial 2.

TABLE 2

RETENTION OF AN ESTABLISHED PLUS-MAZE RETEST-EFFECT IN INTACT MALE SWISS WEBSTER MICE (n = 48).

	Trial 1		Trial 2		Trial 3		Trial 4	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Total entries	22.42	1.05	18.69‡	1.00	16.44‡	1.07	14.96‡	1.11
Open entries	8.17	0.70	4.77‡	0.62	4.83‡	0.72	5.02‡	0.75
Closed entries	14.25	0.69	13.92	0.65	11.60‡¶	0.76	9.94†§	0.72
%Open entries	35.07	2.25	22.13‡	2.40	25.35†	3.12	28.14*	3.13
%Open time	18.83	1.42	10.02‡	1.26	8.44‡	1.24	7.57‡	1.18
%Closed time	27.05	1.07	39.12‡	2.22	49.45‡#	3.40	52.23‡	3.59
%Centre time	54.11	1.41	50.86	1.91	42.11‡#	2.79	40.20‡	2.96
Rears	12.17	1.15	10.88	1.08	11.25	1.05	11.73	1.24
Rearing (duration)	7.19	0.97	8.50	1.05	10.30*	1.29	9.63	1.25
Head-dips	27.79	1.38	12.31‡	1.39	12.77‡	1.57	10.04‡	1.24
%Protected head-dips	69.28	2.57	86.45‡	2.24	88.33‡	2.85	80.38‡¶	3.46
Stretched-attend postures	51.02	2.56	37.81‡	1.84	27.00‡#	1.40	23.83‡	1.47
%Protected SAPs	83.28	1.45	91.43‡	1.18	92.67‡	92.67	90.15‡	1.64
Closed arm returns	1.48	0.42	3.02*	0.60	1.73¶	0.42	1.13	0.34
Sniffing (duration)	97.80	4.96	133.08‡	6.29	160.53‡#	5.11	131.89‡#	5.51
%Protected sniffing	83.80	1.70	92.53‡	1.23	93.50‡	93.50	94.84‡	0.97
Flat-back approach (duration)	10.93	0.68	11.92	0.92	12.11	1.13	8.39¶	0.87
%Protected flat-back	36.66	3.35	46.02	5.26	45.16	45.16	42.46	5.58
Grooming (duration)	3.47	0.62	7.65	1.25	12.64‡	2.49	26.34‡#	3.39

Intertrial interval for Trials 1–3 = 24 h. Intertrial interval between Trials 3 and 4 = 10 days. SAP = Stretched attend postures.
Post-hoc analyses (Newman–Keuls comparisons); *$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ vs. trial 1; §$p < 0.05$, ¶$p < 0.01$, #$p < 0.001$ vs. preceding trial.

RESULTS

Effect of Repeated Test Experience on Gross Behavioral Profiles

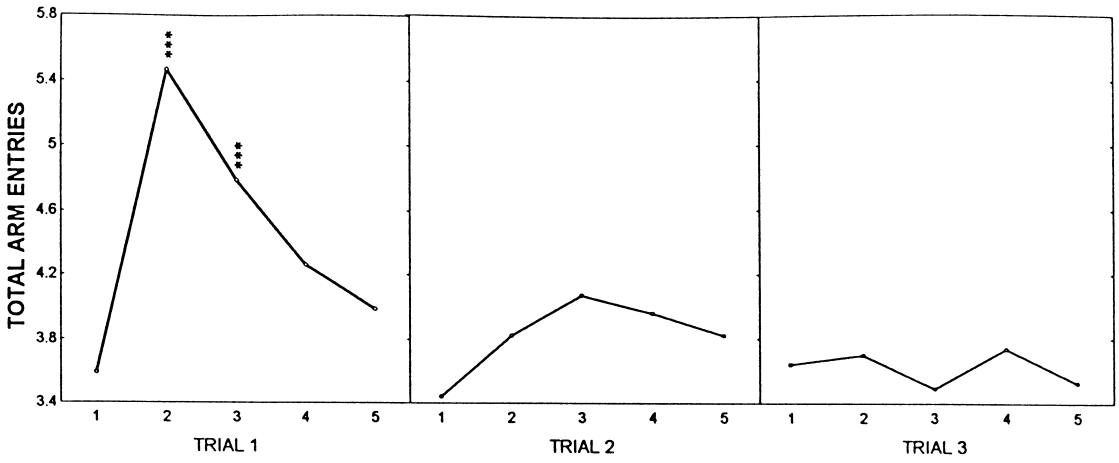
Table 1 summarizes the changes in gross behavioral profiles observed over three successive trials in the plus-maze. ANOVA revealed that the vast majority of behavioral measures were significantly altered by prior maze experience [all $F(2, 146) > 3.89$, $p < 0.025$]: total entries, open-arm entries, closed-arm entries, % open entries, % open time, % closed time, % center time, rear duration, head dips, stretched attend postures, closed-arm returns, sniff, groom, and the % protected forms of SAP, sniff, and head dipping. Measures not affected by prior experience were rear frequency, flatback approach, and % protected flatback approach. Further analysis (Table 1) indicated that, relative to trial 1, behavioral profiles on trial 2 comprised (a) reductions in total arm entries, open entries, % open entries, % open time, % center time, head dips, and SAP, together with (b) increases in % closed time, sniffing, grooming, closed-arm returns, and the protected forms of head dipping, SAP, and sniffing. Table 1 also shows that the changes observed on trial 2 were either fully maintained or further strengthened (i.e., total entries, % closed time, % center time, sniff, groom, and SAP) on trial 3. Significantly, closed-arm entries did not change between trials 1 and 2, but, rather, showed a significant decline by trial 3.

To assess the persistence of this retest effect, a subset of the present sample (n = 48) was exposed to the maze for a fourth time 10 days following trial 3 (Table 2). With the exception of rear frequency and % protected flatback approach which (as above) were unaltered by prior maze experience, ANOVA on this smaller database confirmed the potent effects of prior experience on all other behavioral measures [all $F(3, 141) > 3.26$, $p < 0.025$]. Follow-up comparisons (Table 2) revealed a virtually identical pattern of behavioral change over the first three trials to that seen for the full dataset (Table 1). Furthermore, this analysis demonstrated the enduring nature of prior maze experience in that the behavior of animals on trial 4 remained significantly different to their naive (trial 1) profile but did not differ substantively from their trial 3 profile.

Minute-by-Minute Changes Within and Between Plus-Maze Trials

To examine more closely how behavior changed both within and between trials, the full dataset presented in Table 1 (i.e., three trials, n = 74) was broken down into 1-min time bins (Figs. 1–5). Two-way ANOVA revealed a large number of significant trial × time bin interactions indicating that, for these variables, the within-session pattern of behavioral change differed as a function of test trial [all $F(8, 568) > 1.96$,

FIG. 1. Minute-by-minute changes in total arm entries, closed-arm entries, open-arm entries, and percent open-arm entries for male Swiss–Webster mice over three successive plus-maze trials (intertrial interval, ITI = 24 h). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. minute 1 of same trial. °$p < 0.05$, °°$p < 0.01$, °°°$p < 0.001$ vs. minute 1 of previous trial. $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ vs. minute 5 of previous trial. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. minute 1 of trial 1.


TOTAL ARM ENTRIES
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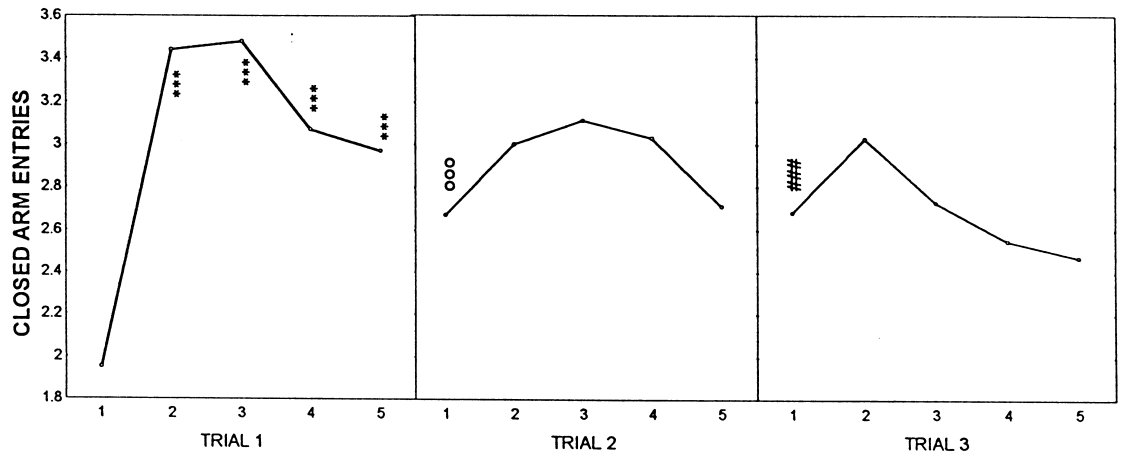
5.4
5
4.6
4.2
3.8
3.4
***
***
1 2 3 4 5
TRIAL 1
1 2 3 4 5
TRIAL 2
1 2 3 4 5
TRIAL 3


CLOSED ARM ENTRIES
3.6
3.4
3.2
3
2.8
2.6
2.4
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2
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1 2 3 4 5
TRIAL 1
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TRIAL 2
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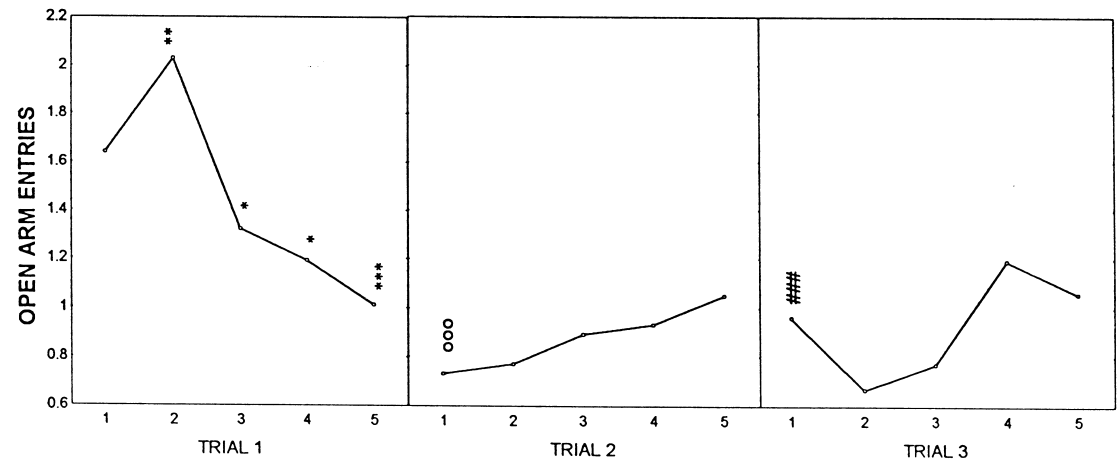
TRIAL 3


OPEN ARM ENTRIES
2.2
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1.4
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1
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TRIAL 1
1 2 3 4 5
TRIAL 2
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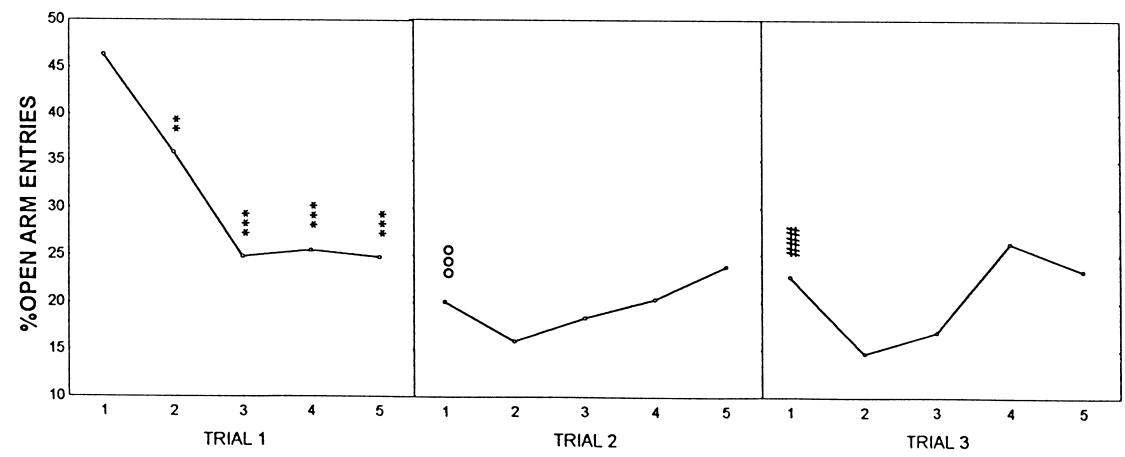
TRIAL 3


%OPEN ARM ENTRIES
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45
40
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TRIAL 1
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TRIAL 2
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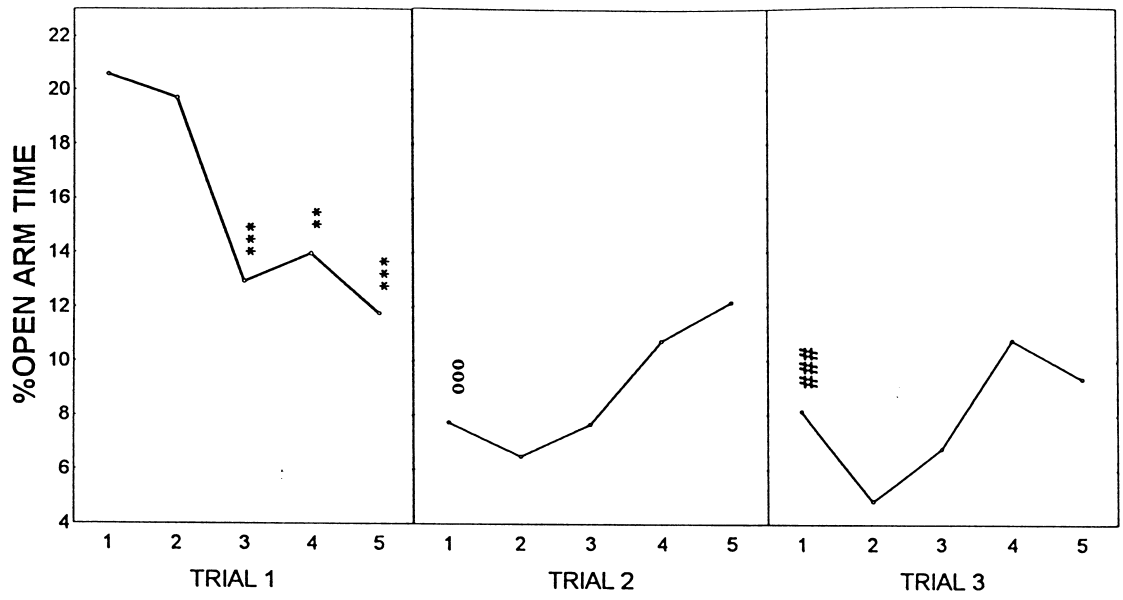
TRIAL 3


%OPEN ARM TIME
22
20
18
16
14
12
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6
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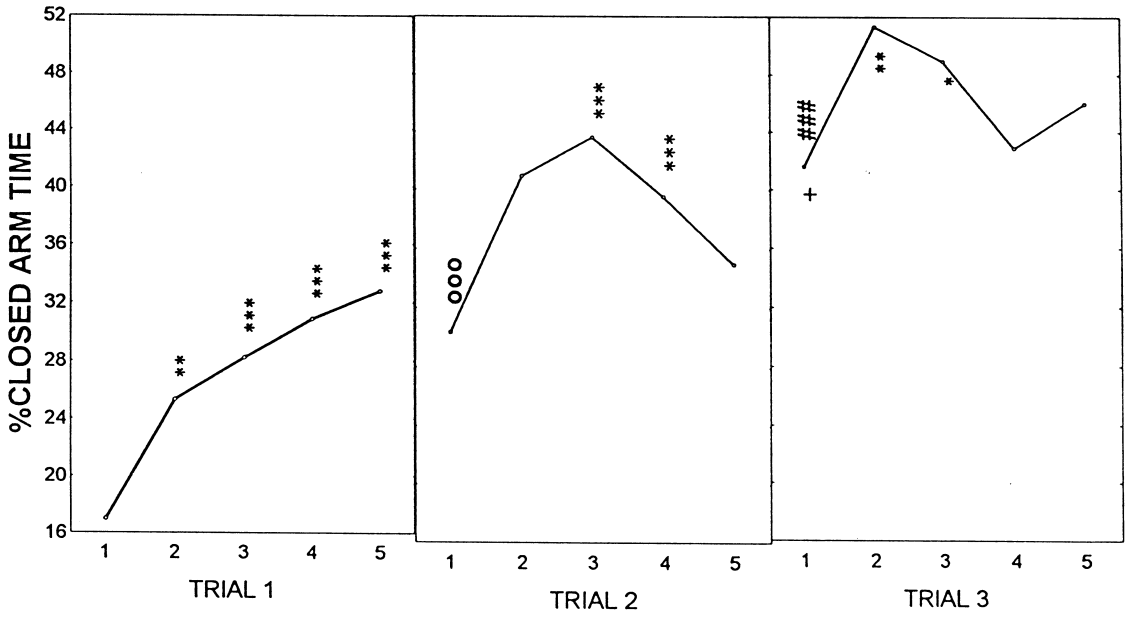
1 2 3 4 5
TRIAL 1
TRIAL 2
TRIAL 3


%CLOSED ARM TIME
52
48
44
40
36
32
28
24
20
16
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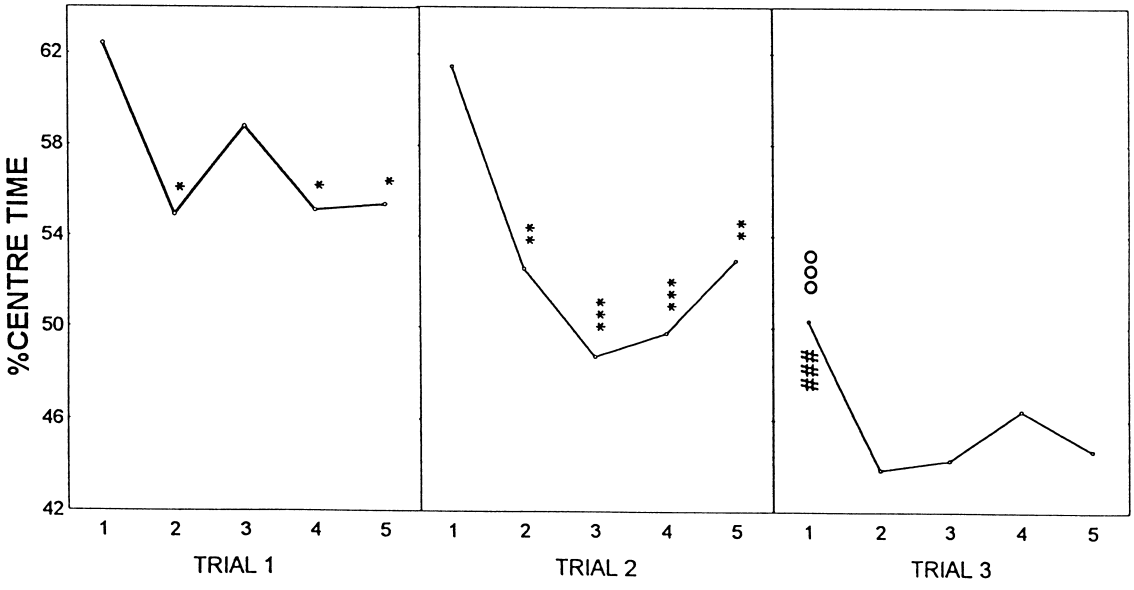
+
1 2 3 4 5
TRIAL 1
TRIAL 2
TRIAL 3


%CENTRE TIME
62
58
54
50
46
42
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1 2 3 4 5
TRIAL 1
TRIAL 2
TRIAL 3

$p < 0.05$]: total entries, open entries, closed entries, % open entries, % open time, % closed time, rear duration, closed-arm returns, head dips, SAP, sniff, % protected SAP, and % protected flatback approach. Significant main effects (but no interactions) of trial (df = 2,142) and time bin (df = 4,284) were observed for % center time, $F = 12.91$, $p < 0.001$; $F = 7.18$, $p < 0.001$, and grooming, $F = 15.92$, $p < 0.001$; $F = 5.17$, $p < 0.001$, demonstrating that, although these measures changed both within and between trials, the pattern of within-session change was similar on each occasion. Significant main effects of trial only for % protected head dips, $F(2, 40) = 7.06$, $p < 0.001$, and % protected sniff, $F = 10.55$, $p < 0.001$, indicated that these measures differed between but not within trials, while significant effects of time bin only for rear frequency, $F = 38.20$, $p < 0.001$) and % protected flatback, $F(4, 60) = 31.44$, $p < 0.001$, indicated that these measures differed within but not between trials.

Further analysis (Figs. 1–5) showed that during the first minute of trial 1, animals spent most of their time on the center platform (>60%) with the remaining time divided equally (ca. 20%) between closed and open arms: there were correspondingly few arm entries and these were also equally distributed between open and closed sections. However, while rearing, sniffing, and grooming were infrequent, this initial period was characterized by high levels (absolute and protected) of head dipping, SAP, sniffing, and flatback approach. By the second minute, center time had decreased and closed time increased; this change was accompanied by large increases in closed arm entries and rearing, small (though significant) increases in open-arm entries and % protected SAP, and decreases in % open entries, head dipping, SAP, sniffing, and % protected flatback. From minute 3 to minute 5, closed entries remained high, closed time and rearing increased further, and open entries, % open entries, open time, center time, head dips, and SAP decreased further. It is notable that % protected SAP increased from the second to third minute but thereafter declined to levels significantly lower than those observed in the first minute. Generally speaking, the behavior pattern observed by the final minute of trial 1 was either maintained or further enhanced on subsequent trials (Figs. 1–5). However, it is pertinent to note that in the first minute of each retest trial, SAP, closed arm returns, sniffing, % protected SAP, and % protected flatback showed significant increases relative to the final minute of the preceding trial, whereas rearing showed a relative decrease.

Factor Analysis

Trial 1 only: all measures (Table 3). Factor analysis of all T1 behavioral measures yielded a six-factor structure accounting for 83.84% of total variance. Factor 1 (37.01%) gave high positive loadings for the conventional measures of open-arm avoidance as well as total entries, and was associated with high negative loadings for % center time and all % protected scores. Factor 2 (18.56%) loaded highly for closed arm entries and more moderately for total entries, closed-arm returns, and stretched attend postures. Factor 3 (9.27%) loaded highly but in opposite directions for sniffing and head dipping. Factor 4 (7.69%) loaded highly for rearing. Factor 5 (5.97%) for grooming and % closed arm time. Factor 6 (5.32%) for flatback approach.

All trials: conventional measures only (Table 4). Factor analysis limited to conventional scores from all three trials revealed a four-factor structure accounting for 80.13% of the total variance. Factor 1 (41.36%) predominantly loaded for T2 and T3 measures of % open time, % open entries, and open entries, although moderate loadings for T2 and T3 measures of total entries and % closed time (-ve) were also apparent. Factor 2 (17.49%) showed strong loadings for T2 and T3 measures of closed entries and total entries. Factor 3 (12.37%) loaded strongly for T1 measures of open entries, % open time, % open entries, and total entries. Factor 4 (8.91%) comprised strong loadings for T1 closed entries and % closed time, a high loading for T2 % closed time, and a moderate loading for T1 total entries.

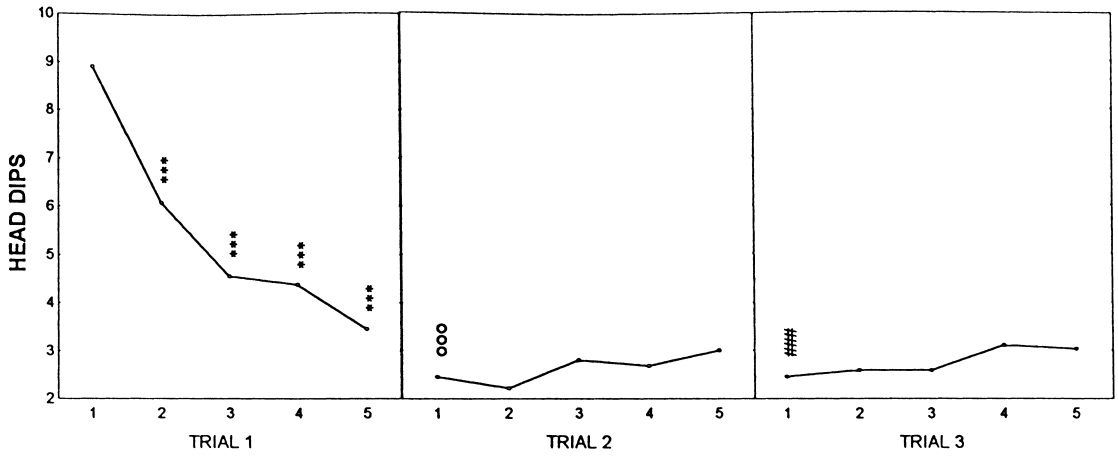
All trials: conventional measures plus center time (Table 5). The addition of scores for % center time yielded a five-factor structure that accounted for 83.85% of total variance. Factor 1 (36.95%) gave high loadings for closed and total entries from T2 and T3, while factor 2 (18.62%) primarily loaded for T1 measures of closed entries, closed time, and total entries. Factor 3 (12.49%) loaded highly for T1 measures of % open time, open entries, % open entries, % center time (-ve), and total entries. Factor 4 (9.64%) comprised high loadings for T2 and T3 measures of % open time, % open entries, open entries, and moderate loadings for T2 and T3 total entries. Factor 5 (6.14%) loaded highly for T2 and T3 measures of % center time and closed time (-ve).

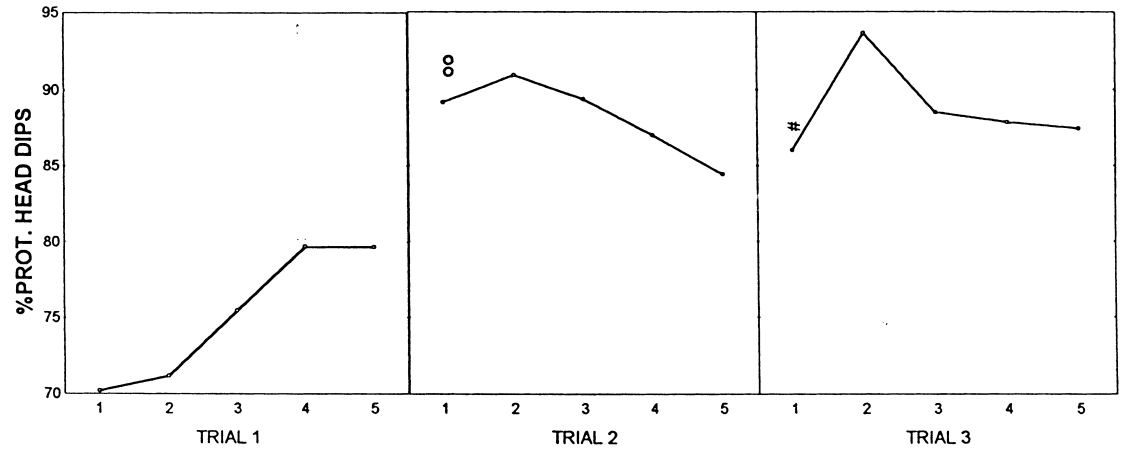
All trials: conventional and ethological measures (Table 6). Although analysis of the full dataset for all three trials was precluded by an unfavorable subject:variable ratio (9,30), it was considered potentially informative to run a final factor analysis incorporating some ethological measures. The data entered into the final factor analysis were based on the need to (a) maintain a respectable ratio of subjects to variables (>3:1), and (b) include those high probability behavioral measures most frequently recorded in ethological studies involving the plus-maze (i.e., rearing and head dipping). This final analysis (Table 6) yielded a six-factor structure accounting for 83.91% of the total variance. Factor 1 (36.08%) gave high loadings for T2 and T3 measures of open entries and % open time, although moderate loadings for T2 and T3 total entries were also obtained. Factor 2 (16.43%) loaded highly and exclusively for rearing on all three trials. Factor 3 (10.24%) loaded strongly for T2 and T3 measures of total entries and closed entries, while factor 4 (8.48%) loaded most strongly for T1 measures of open-arm entries and % open-arm time. Factor 5 (7.19%) loaded highly and exclusively for head dips on all three trials, and factor 6 (5.49%) yielded high loadings for T1 measures of closed entries and total entries.

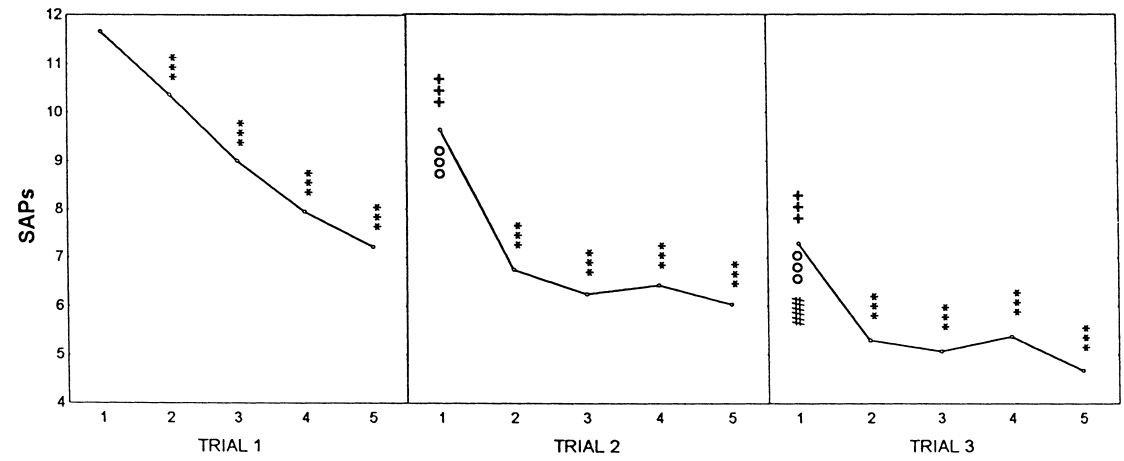
DISCUSSION

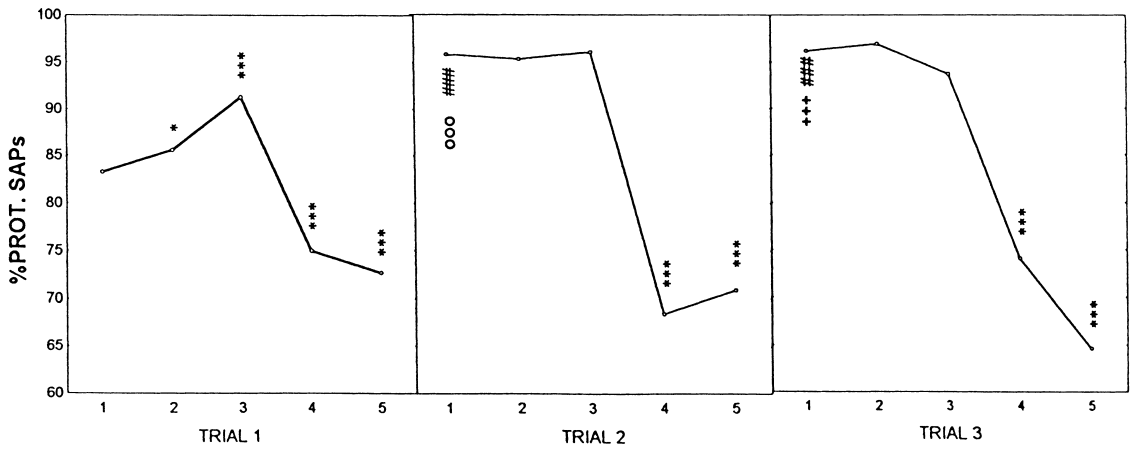
In an extensive investigation of the plus-maze performance of 16 inbred mouse strains, Trullas and Skolnick (53) found that over 70% of the variance in open-arm activity measures could be attributed to genetic factors. Using a derived index of plus-maze responsivity, they were able to categorize strains into four distinct groups: nonreactive (e.g., BALB/c), intermediate-low reactive (e.g., C3H.SW/SnJ), intermediate-high reactive (e.g., DBA/2J), and high reactive (e.g., C57BL/6J). Results obtained with inbred strains in our own laboratory are

FIG. 2. Minute-by-minute changes in percent open-arm time, percent closed-arm time, and percent center platform time scores for male Swiss–Webster mice over theee successive plus-maze trials (ITI = 24 h). For key to symbols, see legend for Fig. 1.


HEAD DIPS
TRIAL 1
TRIAL 2
TRIAL 3


%PROT. HEAD DIPS
TRIAL 1
TRIAL 2
TRIAL 3


SAPs
TRIAL 1
TRIAL 2
TRIAL 3


%PROT. SAPs
TRIAL 1
TRIAL 2
TRIAL 3

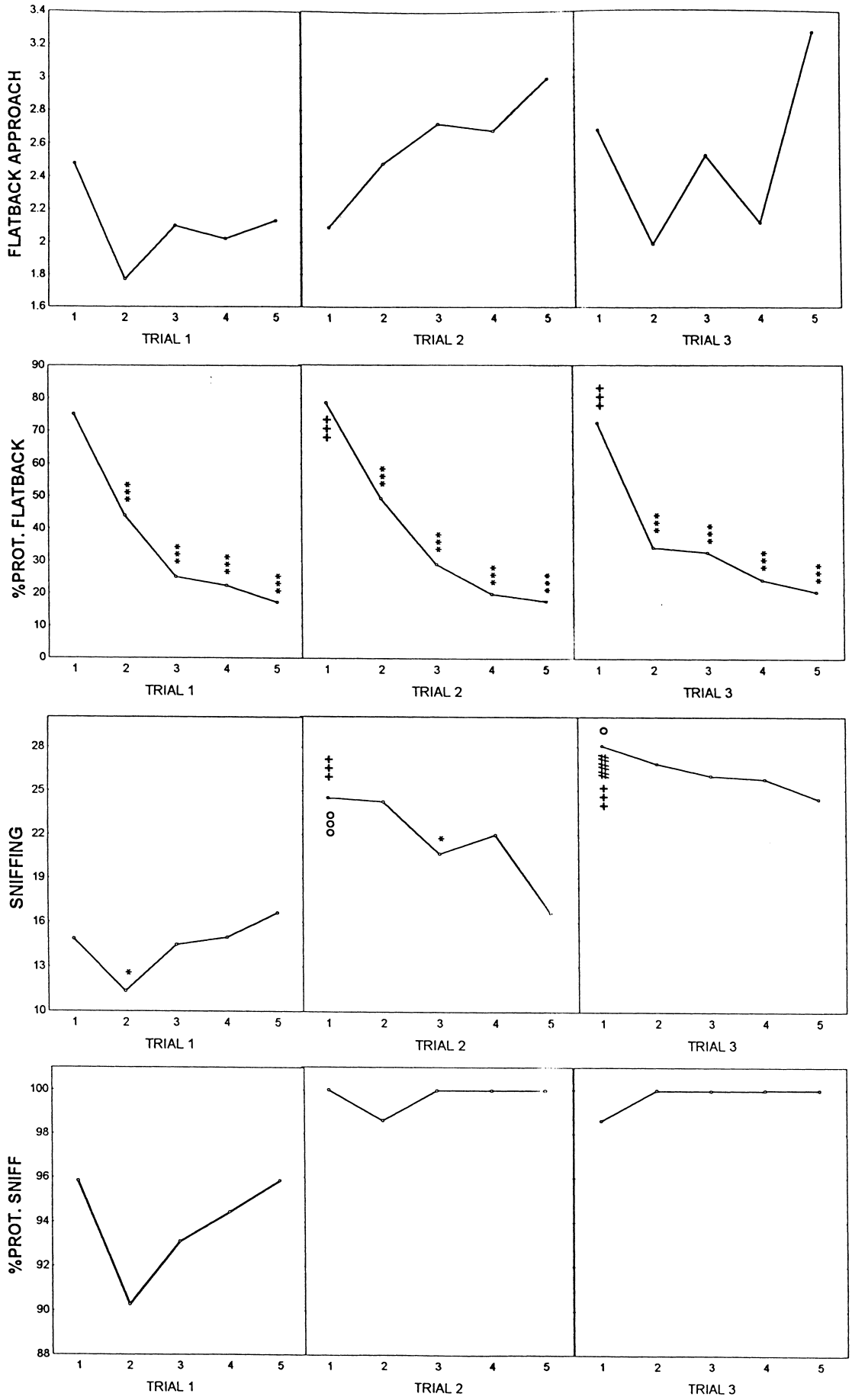
generally consistent with these observations: thus, DBA/2 mice characteristically show low levels open arm activity (% open time ca. 10%) while, if anything, animals of the BALB/c strain actually avoid the enclosed arms (% open time ca. 75%) (10). Relative to these profiles, two outbred strains (Tuck-1 and CD-1) were found to be much more active in the maze and to display intermediate profiles of open-arm avoidance (% open time ca. 20 and 30%, respectively) (10,39,42). In general agreement with these observations, present results (Table 1) indicate that test-naive Swiss–Webster mice are very active in the plus-maze, showing particularly high levels of head dipping, stretched attend postures, and sniffing. Furthermore, the spatiotemporal distribution of their behavior on the maze (% open entries; % time) is very much more similar to that of outbred Tuck-1 and CD-1 strains than the inbred DBA/2 strain (10,42). Thus, unlike the typical DBA/2 profile of closed time > center time > open time, but similar to findings reported for the C3H.SW/SnJ (53) and CD-1 (42) strains, maze-naive Swiss–Webster mice display a rank order preference of center time > closed time > open time.

Despite these strain differences in initial response patterns, present findings confirm that prior maze experience produces major changes in the way in which rodents react to this test (2,13,15,24,25,31,44–48,52). On first reexposure (T2), significant reductions were observed in total arm entries, open entries, % open entries, % open time, % center time, head dipping, and stretched attend postures. These changes were accompanied by significant increases in % closed time, rear duration, closed-arm returns, sniffing, grooming, and the % protected forms of head dipping, SAP, and sniffing (Table 1). As no alterations in closed-arm entries were apparent, this retest profile is more consistent with anxiety enhancement/fear sensitization [e.g., (21,23,45,46,52)] than with locomotor habituation (13). This interpretation is also supported by reference to the effects of anxiogenic compounds [e.g., (12)], which, with the principal exception of reducing closed-arm entries, produce effects comparable to those seen in plus-maze–experienced animals. Indeed, the reduction in overall activity levels with proanxiety compounds such as PTZ and picrotoxin suggests that these agents produce a more global increase in fear than that seen in response to a single prior experience of the maze. Importantly, the behavioral changes observed on T2 were either maintained or further enhanced on T3 [see also, (15)], with a very similar pattern evident when a subsample was tested again some 10 days later (Table 2). However, despite the absence of a reduction in locomotor activity between T1 and T2, it is relevant to note that significant decreases in closed-arm entries were evident by T3 and T4. This observation closely parallels recent findings in Lister hooded rats (15) and, in line with the above argument, would be consistent with yet further fear enhancement.

These profound effects of repeated testing confirm the potency of prior experience in altering subsequent reactions to the plus-maze. Although previous findings suggest that such learning is unaffected by between-test variations in maze orientation/location/construction (20,44), it has been argued that initial experience of open arms is a crucial factor (20). The observation that significant test-retest changes are apparent even when the duration of T1 is limited to 2 min (45) would not be inconsistent with this proposal, especially in view of evidence that during the first minute of T1 animals do not discriminate between open and closed arms but thereafter show a substantial within-session increase in open-arm avoidance (45,48). The results of the present time bin analyses extend these earlier findings to subsequent trials (Figs. 1-5) and, in accordance with previous findings in DBA/2 mice (45), show clear time-dependent behavioral changes within T1. Thus, from a first-minute baseline of roughly equal entries into and time spent on open and closed arms, mice rapidly shifted their spatial preference to the enclosed parts of the maze. This alteration in the spatiotemporal distribution of behavior was accompanied by major changes in the frequency and/or duration of specific behavioral acts and postures, with increases in closed-arm–related behaviors (e.g., rearing) and reductions in open-arm/center platform behaviors (e.g., head dipping and SAP). Interestingly, the percent protected form of SAP was the only measure to show a biphasic profile within T1, increasing in minutes 2–3 and decreasing in minutes 4–5. In agreement with earlier findings in rats (48), the behavioral changes present by the end of the first trial were generally maintained or further enhanced on T2 and T3. Thus, mice commenced each new trial with a behavioral profile very similar to that with which they ended the preceding session, i.e., within-session learning (particularly in T1) readily generalized between sessions. However, it is particularly interesting to note that, relative to the final minute of the preceding trial, the first minutes of T2 and/or T3 were characterized by a "reinstatement" of higher levels of SAP, sniffing, closed-arm returns, and % protected forms of head dipping, SAP, and sniffing (as well as lower levels of rearing). This profile would not be inconsistent with an initial and rapid refamiliarization with the spatial configuration of the maze prior to reversion to a typical retest preference for the closed arms. Together, these novel findings support previous suggestions (45–47) that prior test experience not only alters gross plus-maze behavioral profiles but also results in an altered behavioral strategy.

In recent years, factor analytic methods have been increasingly used in anxiety research, both to identify commonalities and differences between animal models of anxiety [e.g., (3,8,17,37)] and to characterize relationships among specific indices within the same model. In the latter context, findings based on conventional plus-maze T1 scores have been remarkably consistent across species and strain, with measures of open arm avoidance ("anxiety") and closed arm entries ("activity") loading on independent factors (11,14,15,17,32,33,43,53). Furthermore, the inclusion of absolute or percent center time scores has often [e.g., (11,43,53)], although not always [see (15,33)], revealed a third factor thought to reflect "decision making." When applied to databases comprising both conventional and ethological measures, factor analyses have uncovered yet further dimensions to plus-maze behavior patterns. For example, although employing rather different behavioral measures and/or different behavioral definitions, research on male rats (Wistar, hooded Lister) and mice (Swiss) has typically identified a four-factor structure with all reports agreeing on factors related to "anxiety" and "activity" but differing with respect to the composition and/or naming of the remaining factors [e.g., "decision making" and "displacement" (11); "risk assessment" and "impetuosity" (33); "decision height" and "decision open" (15); "approach–avoid con-

FIG 3. Minute-by-minute changes in head dips, percent protected head dips, stretched attend postures (SAP), and percent protected SAP for male Swiss–Webster mice over three successive plus-maze trials (ITI = 24 h). For key to symbols, see legend for Fig. 1.


FLATBACK APPROACH
3.4
3.2
3
2.8
2.6
2.4
2.2
2
1.8
1.6
1 2 3 4 5
TRIAL 1
TRIAL 2
TRIAL 3
%PROT. FLATBACK
90
80
70
60
50
40
30
20
10
0
***
+++
SNIFFING
28
25
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19
16
13
10
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%PROT. SNIFF
100
98
96
94
92
90
88

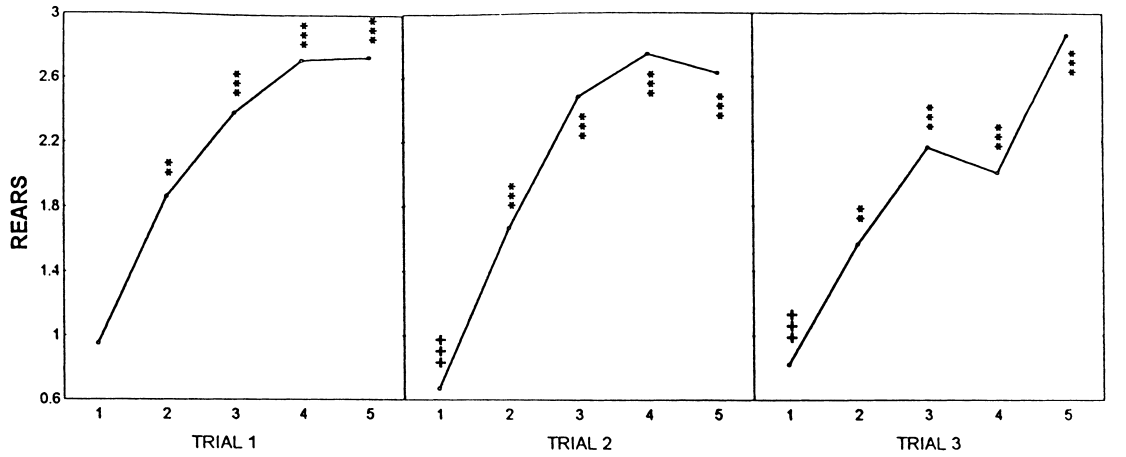
flict" and "displacement" (14)]. Similar findings have been reported for DBA/2 mice (43), but with a six-factor structure comprising: "anxiety" (primarily open-arm avoidance measures including % protected measures), "locomotor activity" (primarily closed entries), "risk assessment" (SAP, sniffing), "decision making" (primarily center time), "vertical activity" (rearing), and "directed exploration" (head dipping). Although variation in the number of independent factors identified by these studies may simply be a consequence of methodological differences (e.g., the number and type of behavioral measures recorded), present findings suggest that strain differences cannot be ignored.

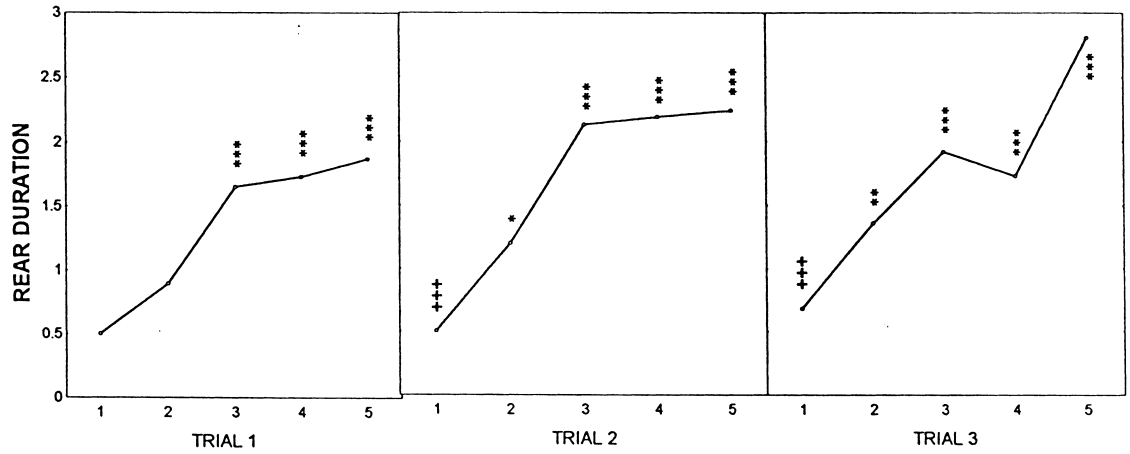
Thus, while factor analysis of the trial 1 plus-maze profile of Swiss–Webster mice (Table 3) also produced a six-factor structure, the composition of these factors differed in several important ways to that previously reported for DBA/2 mice (43). Factor 1, loading for conventional measures of open-arm avoidance and the precent protected scores, is clearly related to "anxiety"; however, unlike the DBA/2 profile, this factor also loaded strongly and negatively for % center time. This finding is consistent with the much higher proportion of time center observed in Swiss–Webster mice (discussed above) and suggests that, in this strain, center time reflects anxiety rather than "decision making" [see also (15,33)]. Although the high factor 2 loadings for closed-arm entries and total entries strongly suggest a relationship to "locomotor activity," this factor additionally showed moderate-high loadings for total stretched attend postures and closed-arm returns. This finding also contrasts with the pattern observed in DBA/2 mice, in which stretched attend postures are associated with an independent "risk assessment" factor. However, attention is drawn to a potentially crucial difference in the functional significance of "protected" and "unprotected" forms of this posture. Such a distinction is clearly supported by the strong loading for % protected SAP scores on the main anxiety factor (vs. the loading pattern for total SAP) and is further confirmed by a recent factor analytic study by Espejo (14). Using absolute scores for protected and unprotected SAP, this author found the former to load strongly on "anxiety" and the latter on an independent factor equivalent to the risk assessment factor previously identified in DBA/2 mice (43). These data clearly imply that future studies should separately record and analyze the subcomponents of the total SAP score. It is also relevant to note the equal loadings of total arm entries on factors 1 and 2, confirming the inherent weakness of this measure as an index of general activity [e.g., (11,17,43)]. Factor 3 loaded strongly (and in opposite directions) for head dipping and sniffing, suggesting commonality with "directed exploration" factor identified in DBA/2 mice, while the composition of factor 4 (high loadings for rear frequency and duration) is directly comparable to the "vertical activity" factor in the latter strain. However, further confirming strain differences in the structure of plus-maze patterns, the loading pattern for factor 5 (grooming) would suggest a relationship to "displacement" [see also (11,14)], while the high loading for flatback approach on factor 6 would, in accordance with recent work on antipredator defense (4,5), suggest a relationship to "defensive ambulation." Together, these results demonstrate important strain differences in the pattern of behavioral response to the plus-maze, thereby extending previously reported strain variation in gross behavioral profiles in mice (10,39,42,53).

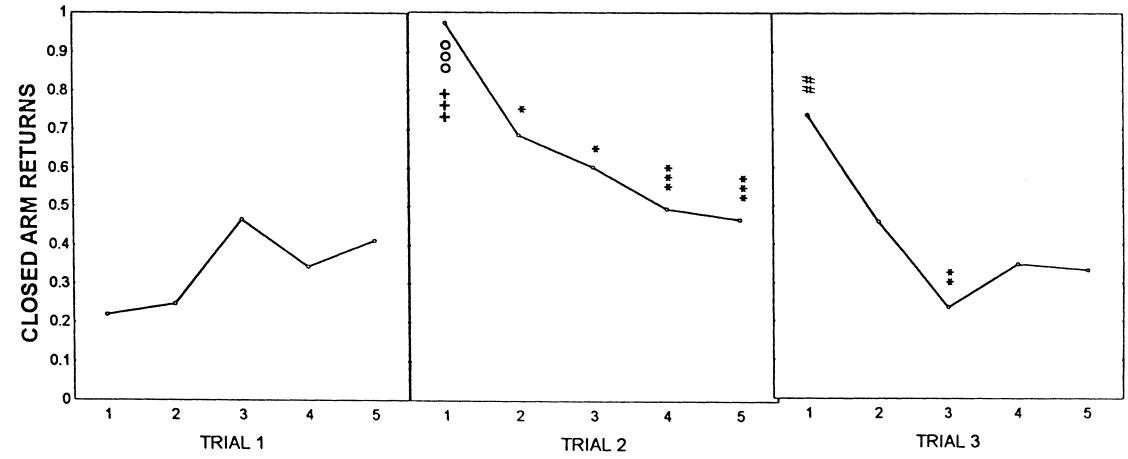
The hypothesis that the nature of anxiety in maze-experienced animals differs fundamentally from that in maze-naive animals would lead to the prediction that anxiety-related measures from different trials should load on independent factors. Consistent with prediction, recent work on the rat plus-maze has shown that: (a) in a two-trial paradigm, T1 and T2 measures of anxiety loaded on separate factors (23), and (b) in a three-trial paradigm, T1 measures of anxiety loaded independently of anxiety measures from T2 and T3 which, together, load on the same factor (15). These results indicate that the shift in emotional response to the maze occurs between the first two trials, and that the state engendered on second trial is largely maintained on further testing. Present data, based the outcome of factor analyses applied to the response profiles of male Swiss–Webster mice on three successive plus-maze trials, confirm the cross-species generality of these findings. Analysis of the conventional measures from all three trials produced a four-factor resolution accounting for >80% of the total variance (Table 4). The loading patterns obtained indicate that factor 1 = "retest (T2/T3) anxiety," factor 2 = "retest (T2/T3) locomotor activity," factor 3 = "naive (T1) anxiety," and factor 4 = "naive (T1) locomotor activity." Irrespective of trial, it is important to note the persistent coloadings of total entry scores on "anxiety" and "locomotor activity" factors, a pattern that differs markedly from the exclusive loadings of closed entry scores on the "locomotor activity" factors. Despite the remarkable similarity between the currently identified four-factor structure for Swiss–Webster mice and that recently documented for Lister hooded rats (15), the latter authors reported absolute dissociations between T1 and T2/T3 behavioral measures. This clearly contrasts with the weak loadings (all <0.50) observed for T2 % closed time on factor 1, T1 closed entries on factor 2, for T2 open entries/% open entries on factor 3, and for T3 % closed time on factor 4, suggesting that the distinction between T1 and T2/T3 behavior patterns in Swiss–Webster mice is somewhat less definitive. However, as Fernandes and File (15) used a loading cutoff of >0.50, and because the application of this higher threshold to present data would also have yielded absolute distinctions between T1 and T2/T3 measures, the apparent discrepancy in findings may simply be ascribed to between-laboratory variation in the arbitrarily defined factor loading threshold.

Inclusion of percent center time scores in the present study (Table 5) yielded a five-factor resolution accounting for approximately 84% of the total variance. Although the new analysis produced a different factor order, loadings for the first four factors map well onto those identified in the initial analysis (factor 1 = "retest locomotor activity"; factor 2 = "naive locomotor activity"; factor 3 = "naive anxiety"; factor 4 = "retest anxiety"). However, whereas T1 % center time loaded negatively on the "naive anxiety" factor, T2 and T3 % center time scores loaded independently, exclusively, and positively on the new factor 5. This is an intriguing finding in that previous studies on the behavioral structure of animals naive to the maze have found this variable to load either on a separate "decision-making" factor (11,43,53), or on the main "anx-

FIG. 4. Minute-by-minute changes in flatback approach (duration, s), percent protected flatback approach, sniffing (duration, s), and percent protected sniffing for male Swiss–Webster mice over three successive plus-maze trials (ITI = 24 h). For key to symbols, see legend for Fig. 1.


REARS
TRIAL 1
TRIAL 2
TRIAL 3


REAR DURATION
TRIAL 1
TRIAL 2
TRIAL 3


CLOSED ARM RETURNS
TRIAL 1
TRIAL 2
TRIAL 3

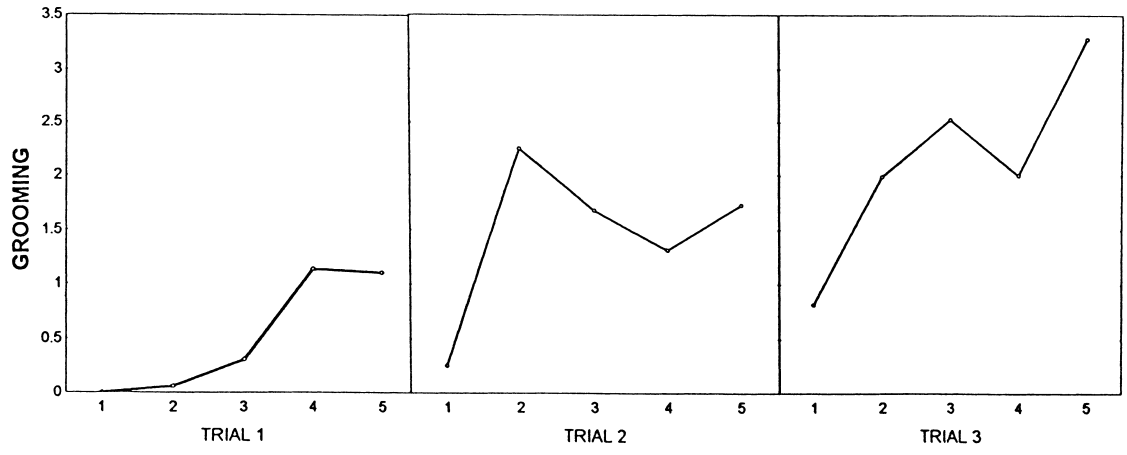

GROOMING
TRIAL 1
TRIAL 2
TRIAL 3

TABLE 3
ORTHOGONAL FACTOR LOADINGS FOR PLUS-MAZE TRIAL 1

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Total entries	0.65	0.64				
Open entries	0.93					
Closed entries		0.89				
%Open entries	0.88					
%Open time	0.97					
%Closed time		0.47		0.44	−0.66	
%Centre time	−0.77				0.41	
Rears				0.91		
Rearing (duration)				0.95		
Head-dips			−0.63			
%Protected head-dips	−0.89					
Stretched-attend postures		0.55				
%Protected SAP	−0.92					
Sniffing (duration)			0.86			
%Protected sniffing	−0.81					
Closed-arm returns	−0.41	0.68				
Flat-back approach (dur.)						−0.86
%Protected flat-back	−0.65					0.46
Grooming (duration)					−0.86	

Conventional and ethological measures (total variance = 83.84%). Factor loadings of <0.4 are not included. See Tables 1 and 4–6 and text. Key: SAP; stretched-attend postures, dur; duration.

iety" (33) or "activity" (15) factors. Current results suggest that, in Swiss–Webster mice, the significance of center time may change as a function of test experience, being positively related to anxiety in naive animals and to decision making in maze-experienced animals. An alternative, and perhaps more parsimonious, interpretation is that the shift in factor loading for % center time is merely a statistical consequence of the reduction in open-arm time. However, in this context, attention has already been drawn to the high T1 % center time scores of naive Swiss–Webster mice vs. other mouse strains (10,53) and to the experientially induced reduction in these scores (this study). In addition to the expected coloadings of total entries and closed entries on the trial-specific anxiety and locomotor activity factors (see above), several other measures also showed loadings on an additional factor (i.e., T1 closed entries and % center time on F2, T2 open entries on F3). However, it is important to emphasize that these were very much weaker than their main factor loadings, i.e., just above the cut-off value of ≤0.40 and, hence, likely to be of little importance.

Ideally, the final factor analysis would have incorporated all variables for each of the three trials. Unfortunately, however, this would have resulted in a subject:variable ratio of <2:1 and, consequently, concern about the reliability of the analysis (9,30). Nevertheless, the pattern of results obtained within and between trials suggested the potential utility of a factor analysis incorporating at least some of the ethological measures. To accommodate this aim, while retaining an acceptable subject:variable ratio, the final analysis was applied to a database comprising key conventional parameters (i.e.,

TABLE 4
ORTHOGONAL FACTOR LOADINGS FOR PLUS-MAZE TRIALS 1, 2, AND 3

	Factor 1	Factor 2	Factor 3	Factor 4
Total entries 1			0.65	−0.53
Total entries 2	0.45	0.71		
Total entries 3	0.51	0.76		
Open entries 1			0.92	
Open entries 2	0.68		0.48	
Open entries 3	0.91			
Closed entries 1		0.43		−0.77
Closed entries 2		0.74		
Closed entries 3		0.92		
%Open entries 1			0.86	
%Open entries 2	0.68		0.44	
%Open entries 3	0.89			
%Open time 1			0.90	
%Open time 2	0.73			
%Open time 3	0.93			
%Closed time 1				−0.77
%Closed time 2	−0.40			−0.66
%Closed time 3	−0.64			−0.40

Conventional measures only (total variance = 80.13%). Factor loadings of <0.4 are not included. See Tables 1, 3, 5, and 6 and text.

FIG. 5. Minute-by-minute changes in rear frequency, rear duration (s), closed-arm returns, and grooming (duration, s) for male Swiss–Webster mice over three successive plus-maze trials (ITI = 24 h). For key to symbols, see legend for Fig. 1.

TABLE 5
ORTHOGONAL FACTOR LOADINGS FOR PLUS-MAZE TRIALS 1, 2, AND 3

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Total entries 1		0.52	0.67		
Total entries 2	0.70			0.47	
Total entries 3	0.75			0.53	
Open entries 1			0.90		
Open entries 2			0.41	0.73	
Open entries 3				0.91	
Closed entries 1		0.78			
Closed entries 2	0.70	0.43			
Closed entries 3	0.92				
%Open entries 1			0.84		
%Open entries 2				0.72	
%Open entries 3				0.85	
%Open time 1			0.91		
%Open time 2				0.79	
%Open time 3				0.92	
%Closed time 1		0.79			
%Closed time 2					−0.79
%Closed time 3				−0.49	−0.79
%Centre time 1		−0.41	−0.76		
%Centre time 2					0.86
%Centre time 3					0.88

Conventional measures plus %center time (total variance = 83.85%). Factor loadings <0.4 are not included. See Tables 1, 3, 4, and 6 and text.

total entries, open entries, closed entries, and % open arm time), plus rear duration and head-dip frequency. The latter measures were selected on the basis of pattern of change within and between trials (Figs. 1–5), their factor loading pattern in naive subjects (Table 3), and their inclusion in plus-maze scoring by several independent laboratories [e.g., (14,15,33,43)]. The resultant analysis (Table 6) confirmed the clear separation of anxiety scores from T1 (factor 4) and T2/T3 (factor 1), as well as the distinction between locomotor activity scores from T1 (factor 6) and T2/T3 (factor 3). Once

TABLE 6
ORTHOGONAL FACTOR LOADINGS FOR PLUS-MAZE TRIALS 1, 2, AND 3

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Total entries 1				0.61		0.71
Total entries 2	0.49		0.60			
Total entries 3	0.59		0.72			
Open entries 1				0.90		
Open entries 2	0.73			0.43		
Open entries 3	0.93					
Closed entries 1						0.90
Closed entries 2			0.67			
Closed entries 3			0.91			
%Open time 1				0.92		
%Open time 2	0.78					
%Open time 3	0.93					
Rearing (duration) 1		0.78				
Rearing (duration) 2		0.85				
Rearing (duration) 3		0.89				
Head-dips					0.85	
Head-dips 2					0.87	
Head-dips 3	0.58				0.63	

Representative conventional measures plus rearing (duration) and head-dips (total variance = 83.91%). Factor loadings of <0.4 are not included. See Tables 1 and 3–5 and text.

again, the coloading patterns observed for T1 total entries (factors 4 and 6), and for T2/T3 total entries (factors 1 and 3) confirm the pitfalls associated with reliance upon this measure as an index of general activity. However, by far the most interesting aspect of this analysis concerns rearing and head dipping, which not only formed independent factors [factor 2/ "vertical activity"; factor 5/ "directed exploration"; see (43)] but also retained factor identity across trials. This important finding implies at least some behavioral constancy across repeated plus-maze exposures.

In summary, the present factor analyses demonstrate that although some plus-maze behavioral dimensions (i.e., vertical activity, directed exploration) remain fairly stable across trials, others show a clear demarcation between initial and subsequent trials (e.g., anxiety, locomotor activity). Furthermore, % center time is clearly a measure of anxiety on T1 but may reflect a separate decision-making dimension on retest trials. Together with the other statistical approaches employed in the present study, these data fully support the conclusion that the behavioral state engendered in mice upon reexposure to the plus-maze differs substantially from that engendered on initial exposure. The fact that present findings closely parallel results obtained in the rat plus-maze (15,23), while remarkable in itself, concurs both with the well-established observation that a single undrugged experience of the maze results in a loss of benzodiazepine efficacy in both species (15,16,20,21, 23,32,46,47,52), and the suggestion that prior maze experience produces a qualitative shift in anxiety/fear reactions in this test (23,46,52). This altered state has been linked to the acquisition of a phobic response to the open arms (21,23), a proposal supported by the restoration of benzodiazepine sensitivity when longer test trials (designed to facilitate extinction of the phobic reaction) are employed (18). Although the cross-species generality of this particular finding remains to be determined, present data demonstrate that Swiss–Webster mice very rapidly acquire an open-arm avoidance response during initial exposure to the maze and that, in the absence of major changes in general activity levels, this reaction strongly persists on subsequent reexposure. The observation that the primary anxiety (and activity) indices from naive and subsequent trials load on independent factors serves to further emphasize the impact of prior experience on emotional response to and, hence behavioral strategy employed in future encounters with the plus-maze.

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REFERENCES

1. Adamec, R. E.; Shallow, T.: Lasting effects on rodent anxiety of a single exposure to a cat. Physiol. Behav. 54:101–109; 1993.
2. Almeida, S. S.; Garcia, R. A.; DeOliveira, L. M.: Effects of early protein malnutrition and repeated testing upon locomotor and exploratory behaviors in the elevated plus-maze. Physiol. Behav. 54:749–752; 1993.
3. Belzung, C.; LePape, G.: Comparison of different behavioural test situations used in psychopharmacology for the measurement of anxiety. Physiol. Behav. 56:623–628; 1994.
4. Blanchard, R. J.; Parmigiani, S.; Agullana, R.; Weiss, S. M.; Blanchard, D. C.: Behaviors of Swiss–Webster and C57/BL/6N sin mice in a fear/defense test battery. Aggress. Behav. 21:21–28; 1995.
5. Blanchard, R. J.; Parmigiani, S.; Bjornson, C.; Masuda, C.; Weiss, S. M.; Blanchard, D. C.: Antipredator behavior of Swiss-Webster mice in a visible burrow system. Aggress. Behav. 21:123–136; 1995.
6. Cao, B.-J.; Rodgers, R. J.: Different behavioural profiles of the R(+)- and S(−)-enantiomers of 8-hydroxy-2-(di-n-propylamino)-tetralin in the murine elevated plus-maze. Behav. Pharmacol. 7:810–819; 1996.
7. Cao, B.-J.; Rodgers, R. J.: Anxiolytic-like profile of p-MPPI, a novel 5-HT_1A receptor antagonist, in the murine elevated plus-maze. Psychopharmacology (Berlin) 129:365–371;1997.
8. Chaouloff, F.; Durand, M.; Mormede, P.: Anxiety- and activity-related effects of diazepam and chlordiazepoxide in the rat light/dark and dark/light tests. Behav. Brain Res. 85:27–35; 1997.
9. Child, D.: The essentials of factor analysis, 2nd ed. London: Cassell; 1994.
10. Cole, J. C.; Burroughs, G. J.; Laverty, C. R.; Sheriff, N. C.; Sparham, E. A.; Rodgers, R. J.: Anxiolytic-like effects of yohimbine in the murine plus-maze: Strain independence and evidence against α2-adrenoceptor mediation. Psychopharmacology (Berlin) 118:425–436; 1995.
11. 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.
12. Dalvi, A.; Rodgers, R. J.: GABAergic influences on plus-maze behavior in mice. Psychopharmacology (Berlin) 128:380–397; 1996.
13. Dawson, G. R.; Crawford, S. P.; Stanhope, K. J.; Iversen, S. D.; Tricklebank, M. D.: One-trial tolerance to the effects of chlordiazepoxide on the elevated plus-maze may be due to locomotor habituation, not repeated drug exposure. Psychopharmacology (Berlin) 113:570–572; 1994.
14. Espejo, E. F.: Structure of the mouse behaviour on the elevated plus-maze test of anxiety. Behav. Brain Res. 86:105–112; 1997.
15. Fernandes, C.; File, S. E.: The influence of open arm ledges and maze experience in the elevated plus-maze. Pharmacol. Biochem. Behav. 54:31–40; 1996.
16. File, S. E.: One-trial tolerance to the anxiolytic effects of chlordiazepoxide in the plus-maze. Psychopharmacology (Berlin) 100: 281–282; 1990.
17. 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. London: John Wiley & Sons Ltd; 1992:25–44.
18. File, S. E.: The interplay of learning and anxiety in the elevated plus-maze. Behav. Brain Res. 58:199–202; 1993.
19. File, S. E.; Andrews, N.; Wu, P. Y.; Zharkovsky, A.; Zangrossi, H.: Modification of chlordiazepoxide's neurochemical effects by handling and plus-maze experience. Eur. J. Pharmacol. 218:9–14; 1992.
20. File, S. E.; Mabbutt, P. S.; Hitchcott, P. K.: Characterisation of the phenomenon of "one-trial tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze. Psychopharmacology (Berlin) 102:98–101; 1990.
21. File, S. E.; Zangrossi, H.: "One-trial tolerance" to the anxiolytic actions of benzodiazepines in the elevated plus-maze, or the development of a phobic state? Psychopharmacology (Berlin) 110:240–244; 1993.
22. File, S. E.; Zangrossi, H.; Sanders, F. L.; Mabbutt, P. S.: Raised corticosterone in the rat after expsoure to the elevated plus-maze. Psychopharmacology (Berlin) 113:543–546; 1994.
23. File, S. E.; Zangrossi, H.; Viana, M.; Graeff, F. G.: Trial 2 in the elevated plus-maze: A different form of fear? Psychopharmacology (Berlin) 111:491–494; 1993.
24. Gonzalez, L. E.; File, S. E.: A five minute experience in the elevated plus-maze alters the state of the benzodiazepine receptor in the dorsal raphe nucleus. J. Neurosci. 17:1505–1511; 1997.
25. Griebel, G.; Moreau, J.-L.; Jenck, F.; Martin, J. R.; Misslin, R.: Some critical determinants of the behaviour of rats in the elevated plus-maze. Behav. Proc. 29:37–48; 1993.

26. Griebel, G.; Sanger, D. J.; Perrault, G.: The use of the rat plus-maze to discriminate nonselective and BZ-1 (ω1) selective, benzodiazepine receptor ligands. Psychopharmacology (Berlin) 124:245–254; 1996.
27. Griebel, G.; Sanger, D. J.; Perrault, G.: Genetic differences in the mouse defense test battery. Aggress. Behav. 23:19–31; 1997.
28. Handley, S. L.; Mithani, S.: Effects of alpha-adrenoceptor agonists and antagonists in a maze exploration model of "fear"-motivated behaviour. Naunyn Schmiedebergs Arch. Pharmacol. 327: 1–5; 1984.
29. Hogg, S.: A review of the validity and reliability of the elevated plus-maze as a model of anxiety. Pharmacol. Biochem. Behav. 54:21–30;1996.
30. Kline, P.: An easy guide to factor analysis. London: Routledge; 1994.
31. Lee, C.; Rodgers, R. J.: Antinociceptive effects of plus-maze exposure: Influence of opiate receptor manipulations. Psychopharmacology (Berlin) 102:507–513; 1990.
32. Lister, R. G.: The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berlin) 102:507–513; 1987.
33. McCreary, A. C.; McBlane, J. W.; Spooner, H. A.; Handley, S. L.: 5-HT system and anxiety: Multiple mechanisms in the elevated plus-maze. Pol. J. Pharmacol. 48:1–12; 1996.
34. Nutt, D. J.: The pharmacology of human anxiety. Pharmacol. Ther. 47:233–266; 1990.
35. Parmigiani, S.; Brain, P. F.; Palanza, P.: Ethoexperimental analysis of different forms of intraspecific aggression in the house mouse (*Mus musculus*). In: Blanchard, R. J.; Brain, P. F.; Blanchard, D. C.; Parmigiani, S., eds. Ethoexperimental approaches to the study of behavior. Dordrecht: Kluwer; 1989:418–431.
36. Pellow, S.; Chopin, P.; File, S. E.; Briley, M.: Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14:149–167; 1985.
37. Ramos, A.; Berton, O.; Mormede, P.; Chaouloff, F.: A multiple-test study of anxiety-related behaviours in six inbred rat strains. Behav. Brain Res. 85:57–69; 1997.
38. Rodgers, R. J.; Cao, B.-J.; Dalvi, A.; Holmes, A.: Animal models of anxiety: An ethological perspective. Braz. J. Med. Biol. Res. 30:289–304; 1997.
39. Rodgers, R. J.; Cole, J. C.: Influence of social isolation, gender, strain and prior novelty on plus-maze behaviour in mice. Physiol. Behav. 54:729–736; 1993.
40. Rodgers, R. J.; Cole, J. C.: The elevated plus-maze: Pharmacology, methodology and ethology. In: Cooper, S. J.; Hendrie, C. A., eds. Ethology and psychopharmacology. Chichester: John Wiley & Sons Ltd; 1994:9–44.
41. Rodgers, R. J.; Cole, J. C.; Cobain, M. R.; Daly, P.; Doran, P.; Eells, J. R.; Wallis, P.: Anxiogenic-like effects of fluprazine and eltoprazine in the mouse elevated plus-maze: Profile comparisons with 8-OH-DPAT, CGS12066B, TFMPP and mCPP. Behav. Pharmacol. 3:621–634; 1992.
42. Rodgers, R. J.; Cutler, M. G.; Jackson, J. E.: Behavioural effects in mice of subchronic chlordiazepoxide, maprotiline and fluvoxamine. II. The elevated plus-maze. Pharmacol. Biochem. Behav. 57:127–136; 1997.
43. 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. 52:621–624; 1995.
44. Rodgers, R. J.; Johnson, N. J. T.; Carr, J.; Hodgson, T. P.: Resistance of experientially induced changes in murine plus-maze behaviour to altered retest conditions. Behav. Brain Res. 86:71–77; 1997.
45. Rodgers, R. J.; Johnson, N. J. T.; Cole, J. C.; Dewar, C. V.; Kidd, G. R.; Kimpson, P. H.: Plus-maze retest profile in mice: Importance of initial stages of Trial 1 and response to posttrial cholinergic receptor blockade. Pharmacol. Biochem. Behav. 54:41–50; 1996.
46. Rodgers, R. J., Lee, C.; Shepherd, J. K.: Effects of diazepam on behavioural and antinociceptive responses to the elevated plus-maze in male mice depend upon treatment regimen and prior maze experience. Psychopharmacology (Berlin) 106:102–110; 1992.
47. Rodgers, R. J.; Shepherd, J. K.: Influence of prior maze experience on behaviour and response to diazepam in the elevated plus-maze and light/dark tests of anxiety in mice. Psychopharmacology (Berlin) 113:237–242; 1993.
48. Shepherd, J. K.: Preliminary evaluation of an elevated "zero-maze" as a model of anxiety in laboratory rats. J. Psychopharmacol. 6:A56; 1992.
49. Shepherd, J. K.; Grewal, S. S.; Fletcher, A.; Bill, D. J.; Dourish, C. T.: Behavioural and pharmacological characterisation of the "elevated zero-maze" as an animal model of anxiety. Psychopharmacology (Berlin) 116:56–64; 1994.
50. Stephens, D. N.; Meldrum, B. S.; Weidmann, R.; Schnieder, C.; Grutzner, M.: Does the excitatory amino acid receptor antagonist 2-APH exhibit anxiolytic activity? Psychopharmacology (Berlin) 90:166–169; 1986.
51. Tancer, M. E.; Uhde, T. W.: Social phobia: A review of pharmacological treatments. CNS Drugs 3:267–278; 1995.
52. Treit, D.; Menard, J.; Royan, C.: Anxiogenic stimuli in the elevated plus-maze. Pharmacol. Biochem. Behav. 44:463–469; 1993.
53. Trullas, R.; Skolnick, P.: Differences in fear motivated behaviors among inbred mouse strains. Psychopharmacology (Berlin) 111: 323–331; 1993.