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Plus-Maze Retest Profile in Mice: Importance of Initial Stages of Trial 1 and Response to Post-Trial Cholinergic Receptor Blockade

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RODGERS, R. J., N. J. T. JOHNSON, J. C. COLE, C. V. DEWAR, G. R. KIDD AND P. H. KIMPSON. *Plus-maze retest profile in mice: Importance of initial stages of trial 1 and response to post-trial cholinergic receptor blockade.* PHARMACOL BIOCHEM BEHAV 54(1) 41-50, 1996.—Recent research has shown that a single undrugged prior experience of the elevated plus-maze produces significant behavioural changes upon 24-h retest in rats and mice. Typically, when reexposed to the maze, animals display an increased avoidance of the open arms and a corresponding preference for the enclosed sections of the apparatus. Using ethological analyses, the present series of experiments sought to further characterize this phenomenon in mice and to determine whether or not it involves cholinergic receptor mechanisms. Results confirmed that behaviour during Trial 2 is markedly different to that seen on initial exposure, and that such changes are independent of the duration of Trial 1 (2 vs. 5 min). Retest behavioural changes included reduced entry latencies, reduced open arm entries, less time on the open arms and centre platform, lower levels of exploratory head-dipping, and increased entries into and time spent in the closed arms. The importance to the retest phenomenon of the first few minutes of initial exposure was further suggested by min-by-min analyses of the behaviour of animals naive to the maze. Results showed that behaviour during the first min is characterized by high levels of risk assessment from the centre platform and relatively low, but equal, levels of open- and closed-arm exploration. From min 2 onwards, however, behaviour showed a marked change with increasing open arm/centre platform avoidance, increasing closed-arm preference, and decreasing levels of risk assessment and exploratory head-dipping. Thus, it would appear that this within-session aversive learning transfers between sessions to account for behavioural profiles on retest. Irrespective of the duration of Trial 1 (2 or 5 min), posttrial administration of the muscarinic antagonist, scopolamine (0.1-1.0 mg/kg), failed to significantly alter the behavioural changes seen between trials. Data are discussed in relation to the apparent sensitization of fear produced by plus-maze exposure, its possible relation to phobia acquisition, and the need for further research on underlying mechanisms.

Elevated plus-maze Retest Fear sensitization Learning & memory Ethological analysis Scopolamine Mice

THE ELEVATED plus-maze test is the most widely used of all currently available animal models of anxiety that depend upon the study of spontaneous behaviour (30). It is an example of what Lister (23) described as an ethological model, and has been extensively validated for use with both rats (28) and mice (22). Conventional indices of anxiety in this test are related to the spatiotemporal distribution of behaviour, with particular reference to open-arm avoidance (30). More recent studies have further enhanced the ethological validity and pharmacosensitivity of the procedure through the incorporation of a range of specific behavioural acts and postures, many of which are related to the rodent defensive repertoire (3,17,32, 33,38).

One of the most intriguing aspects of the plus-maze is the finding that prior exposure to the test reduces or abolishes the anxiolytic efficacy of benzodiazepine receptor agonists, such as chlordiazepoxide and diazepam (7,8,9,22,34,35). Such effects, initially described as 'one-trial tolerance,' depend rather critically upon initial open-arm experience, occur with intertrial intervals ranging between 24 h and 2 weeks (7,9,22,34,35) and remain robust, even when different mazes are used on the two trials (9). In agreement with the proposal that the nature of the anxiety state engendered on reexposure may be qualitatively different to that experienced on initial exposure (34), a factor analysis study has shown that anxiety indices in the two trials load on separate factors (10), and biochemical assay has

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revealed that plasma corticosterone levels do not habituate between trials (11). Although other interpretations have been forwarded (e.g., 4), these data are consistent with the suggestion that, during initial exposure, animals acquire a phobic avoidance of the open arms which, in turn, may explain their subsequent insensitivity to the anxiolytic effects of benzodiazepines (10,12).

As some kind of spatial learning is clearly taking place during Trial 1 (9,10,34,35), it would be predicted that behavioural baselines should differ between trials. Although some laboratories have reported stable test-retest profiles (e.g., 7,22,28), most have, indeed, found that rats and mice show significantly reduced open-arm exploration on retest (1,4,16, 21,34,35,38). In a most convincing demonstration of this effect, Treit et al. (41) not only reported that rats increase their avoidance of open arms on retest, but also found no evidence of habituation after 18 daily trials. Furthermore, a single forced exposure to the open arms greatly reduces 24-h retest escape latencies from an open arm and markedly increases time spent in the enclosed arms (18). This evidence suggests that rodents retain a strong memory for the threat posed by the open arms, and is consistent with an experientally induced sensitization of fear (41). Studies on the rat 'zero-maze' have also shown a marked reduction from 50% (min 1) to 5% (min 5) in time spent on the open quadrants during initial exposure, indicating considerable within-session learning (38). The mechanisms underlying such behavioural changes (within and between sessions) are at present unknown but, in respect of proposals by File and Zangrossi (10), may have substantial implications for our understanding of phobia acquisition.

In this context, cholinergic mechanisms have long been implicated in learning and memory (for review, see 2). Of current relevance are those data showing that posttrial administration of anticholinergic agents, such as scopolamine, impairs retention for a variety of tasks, including spatial memory (e.g., 13,14,20,26,27,29,36,37,40). The principal aim of the present series was to determine whether or not posttrial administration of scopolamine would impair retention of Trial 1 experience in the murine elevated plus-maze (i.e., prevent the behavioural changes seen upon retest). A posttrial paradigm was employed in view of the highly disruptive effects of scopolamine pretreatment on plus-maze performance (5,6,31, 38), and the interpretative difficulties that would inevitably arise from pretrial drug administration (15,24). In contrast to earlier work on the test/retest phenomenon, the current studies employed a more ethological approach to behavioural analysis (e.g., 30,32,34,39). In all, three studies were conducted. Experiment 1 assessed the effects of posttrial scopolamine on retest performance using a standard test duration of 5 min. Experiment 2 employed an existing database to delineate min-by-min changes in the behavioural profile of mice exposed to the maze for the first time; in particular, to determine the point at which animals begin to show a clear avoidance of the aversive open arms. On the basis of these data, Experiment 3 essentially replicated the first study with the exception that a 2-min exposure was used for Trial 1.

GENERAL METHOD

Animals

Subjects were 12–15-week-old male DBA/2 mice (Biomedical Services, University of Leeds), housed 10 per cage (45 × 28 × 13 cm) for at least 4 weeks prior to testing. They were maintained under a 12-h reversed light cycle (lights off: 0700 h) in a temperature (21 ± 1°C)- and humidity (50 ± 5%)-controlled environment. Food and drinking water were freely available with the exception of the brief test periods. All mice were experimentally naive.

Drugs

Scopolamine hydrobromide (Sigma, Poole, UK) was dissolved in a saline vehicle and administered IP in a volume of 10 ml/kg. Doses cited refer to the salt.

Apparatus

The elevated plus-maze was a modification of that validated for mice by Lister (22). Two open arms (30 × 5 cm) and two enclosed arms (30 × 5 × 15 cm) extended from a common central platform (5 × 5 cm) making the shape of a plus-sign, and the entire apparatus was raised to a height of 45 cm above floor level. The maze floor was made from black Plexiglas and the side- and end-walls of the enclosed arms were clear Plexiglas. Grip on the open arms was facilitated by a small raised edge (0.25 cm) around their perimeter, and testing was conducted under dim red light (4 × 60 W indirect).

Procedure

All testing was conducted during the mid-dark phase of the LD cycle (i.e., 1000–1400 h). To facilitate adaptation, mice were transported from the holding room to the laboratory at least 1 h prior to testing. Testing commenced by placing an animal on the centre platform of the maze facing an open arm. A 2- or 5-min test duration was employed (see below) and, between subjects, the maze was thoroughly cleaned with damp and dry cloths. All sessions were recorded by an overhead videocamera that was linked to a monitor and VCR in an adjacent laboratory. To avoid disturbance to the animals, the experimenter remained in this room during testing.

Three studies were conducted. In experiments involving scopolamine (Experiments 1 and 3), mice were randomly assigned to treatment conditions (n = 9–10; saline, 0.1 and 1.0 mg/kg scopolamine HBr) prior to Trial 1. Doses were selected on the basis of previous findings on the amnestic effects of scopolamine (e.g., 13,14,19,20,25,26,29,36,37,39), and the sensitivity of DBA/2 mice to this muscarinic antagonist (31). Treatments were administered immediately following this test, with mice individually tail-marked (for identification) prior to return to home cages. Retesting (Trial 2) took place 24 h later. For Experiment 1, a conventional 5-min test duration was employed for both trials. In Experiment 3, the duration of Trial 1 was shortened to 2 min and, although Trial 2 was kept at a standard 5-min duration, data were obtained both for the initial 2-min period and the entire 5-min session. Also, in this study, a conventional 5-min(T1)/5-min(T2) saline control condition was run to provide comparator data. Experiment 2 employed our computer database, holding information on behavioural profiles of 125 control, maze-naive (i.e., Trial 1 equivalent) male DBA/2 mice. This database was examined with regard to temporal (i.e., minute-by-minute) changes in behaviour during a conventional 5-min test run.

Behavioural Analysis

Videotapes (Experiments 1 & 3) were scored by highly trained observers (inter- and intra-rater reliability >0.9), who remained blind to treatment conditions until all data had been collected. Tapes were scored for conventional and ethologically derived parameters (30–35). Conventional measures were

TABLE 1

EFFECTS OF SCOPOLAMINE (0.1-1.0 mg/kg, IP), ADMINISTERED IMMEDIATELY AFTER TRIAL 1 (5-min), ON THE 24 h RETEST (5-min) BEHAVIOURAL PROFILE OF MALE DBA/2 MICE
Scopolamine HBr

Behaviour	Saline/T1	Saline/T2	0.1 mg/kg/T1	0.1 mg/kg/T2	1.0 mg/kg/T1	1.0 mg/kg/T2
Total entries	19.2 ± 1.4	21.2 ± 2.3	19.4 ± 1.9	21.2 ± 2.5	16.5 ± 1.9	19.0 ± 1.7
Open entries	6.1 ± 0.6	5.1 ± 1.4	7.7 ± 1.0	5.6 ± 1.7	4.9 ± 0.9	3.1 ± 0.7
Closed entries	13.1 ± 1.2	16.1 ± 1.1	11.8 ± 1.3	15.8 ± 1.9	11.6 ± 1.2	15.9 ± 1.4
% open entries	33.0 ± 2.5	20.6 ± 4.3	38.7 ± 3.6	24.4 ± 5.9	27.6 ± 3.5	15.9 ± 2.8
% open time	14.9 ± 2.4	8.1 ± 1.9	17.6 ± 2.7	10.9 ± 3.6	11.9 ± 3.0	4.7 ± 1.4
% centre time	36.5 ± 2.7	29.0 ± 2.3	33.6 ± 3.0	22.8 ± 1.4	38.8 ± 5.2	24.3 ± 2.1
% closed time	48.6 ± 3.7	62.9 ± 3.6	48.8 ± 2.6	66.3 ± 3.4	49.3 ± 4.3	71.0 ± 3.1
Entry latency	8.8 ± 2.4	4.5 ± 2.2	5.1 ± 1.5	1.6 ± 0.7	16.0 ± 8.0	2.2 ± 0.6
NEB	30.1 ± 4.7	43.0 ± 10.5	32.2 ± 5.7	45.0 ± 7.6	38.0 ± 6.5	70.3 ± 8.3
Rears	10.9 ± 1.2	8.4 ± 1.2	11.9 ± 1.6	15.1 ± 2.7	10.9 ± 1.2	12.0 ± 1.4
Head dips	4.9 ± 0.8	1.8 ± 0.6	5.8 ± 1.1	2.2 ± 1.1	6.1 ± 0.9	1.2 ± 0.3
% *p* Dips	58.2 ± 10.0	48.0 ± 16.1	51.8 ± 12.9	48.9 ± 16.7	59.2 ± 10.2	45.0 ± 15.7
SAP	25.2 ± 1.9	18.0 ± 1.8	22.8 ± 3.0	17.7 ± 2.6	24.6 ± 2.8	15.3 ± 1.0
% *p* SAP	62.7 ± 5.4	76.1 ± 6.3	61.9 ± 7.2	65.0 ± 9.5	72.0 ± 7.3	87.1 ± 5.0
Closed returns	0.3 ± 0.2	0.3 ± 0.3	0.0 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.2

See text and Table 2 for associated inferential statistics. T1 = trial 1; T2 = trial 2; NEB = nonexploratory behaviour; SAP = stretched attend postures; % *p* = percent protected.

the number of open- and closed-arm entries (arm entry defined as all four paws into an arm), and time spent on different sections of the maze (including the central platform). These data were used to calculate total arm entries, percent open entries (open/total × 100), and percent time spent in open, centre, and closed sections of the maze (location/300 × 100 for 5-min test; location/120 × 100 for 2-min test).

Other behaviours recorded were entry latency (time taken at start of session to move into an arm), nonexploratory behaviour (combined duration of immobility and grooming), rearing, and head-dipping (exploratory scanning over the sides of the maze). Measures related to the defensive repertoire (risk assessment) were also recorded, and these comprised stretched attend postures (SAP; forward extension of head and shoulders followed by retraction to original position) and closed arm returns (exiting a closed arm with forepaws only and doubling back into the same arm). In view of the importance of thigmotactic cues in the maze (41), head-dipping and stretched attend postures were differentiated by location as either 'protected' (occurring from relative security of closed arms/centre platform) or 'unprotected' (on or from the open arms). Analogous to calculations for open entries/open time, data for head-dipping and stretched attend postures are presented as total frequencies and as percent protected values (% *p* Dips, % *p* SAPS = protected/total × 100).

Statistical Analysis

Data for Experiments 1 and 3 were analysed by one (treatment)- or two (treatment by trials; repeated measures on trials)-factor analyses of variance (ANOVA). Where indicated (significant F-values), further within and/or between groups comparisons were performed using the appropriate error variance terms from the ANOVA summary tables (correlated *t*-tests and Dunnett's *t*-tests, respectively). Data for Experiment 2 (source: computer database) were analyzed by single-factor (time) repeated measures ANOVA, followed by planned comparisons using minute 1 as referent.

RESULTS

Experiment 1

Trial 1 equivalence. Single factor ANOVAs were used to assess between-group differences during Trial 1 (i.e., prior to drug treatment) (Table 1). These analyses confirmed the reliability of the procedure used to randomize allocation of subjects to test conditions, in that no between-group differences were revealed. With 2 and 26 degrees of freedom and an $F_{crit0.05}$ of 3.37, the following F-values were obtained: total entries ($F = 0.91$), open entries ($F = 2.63$), closed entries ($F = 0.47$), percent open entries ($F = 2.90$), percent open time ($F = 1.10$), percent closed time ($F = 0.01$), percent centre time ($F = 1.08$), entry latency ($F = 1.20$), nonexploratory behaviour ($F = 0.54$), rears ($F = 0.18$), head-dips ($F = 0.61$), percent protected head-dips ($F = 0.13$), stretched attend postures ($F = 0.23$), percent protected stretched attend postures ($F = 0.72$) and closed arm returns ($F = 1.0$).

Effects of retesting and posttrial scopolamine. Two-factor analyses of variance were used to assess the effects of posttrial scopolamine on behavioural changes induced by prior exposure to the plus-maze. As shown in Tables 1 (data summary) and 2 (statistical summary), mice showed significant behavioural changes between trials. Retesting resulted in reductions in open-arm entries, percent open entries, percent open-arm time, percent centre time, head-dipping, stretched attend postures, and entry latencies. In addition, closed-arm entries, percent closed time, percent protected SAP, and nonexploratory behaviour were increased. No 'trial' effects were seen for total arm entries, rearing, percent protected head-dipping, or closed-arm returns. Table 2 also shows that these changes were not significantly affected by immediate posttrial scopolamine (0.1-1.0 mg/kg) treatment.

Experiment 2

Data are summarized in Figs. 1-4. All behavioural measures, with the exception of % *p* SAPS and % *p* Dips, showed

TABLE 2

ANOVA SUMMARY STATISTICS FOR EXPERIMENT 1

Behaviour	Drug (df 2,26)	Trials (df 1,26)	Interaction (df 2,26)
Total entries	0.87, NS	2.47, NS	0.03, NS
Open entries	1.97, NS	5.31, $p < 0.05$	0.22, NS
Closed entries	0.28, NS	11.82, $p < 0.005$	0.13, NS
% open entries	2.45, NS	23.19, $p < 0.001$	0.08, NS
% open time	1.80, NS	21.45, $p < 0.001$	0.01, NS
% centre time	1.26, NS	26.26, $p < 0.001$	1.58, NS
% closed time	0.65, NS	52.00, $p < 0.001$	0.78, NS
Entry latency	1.23, NS	5.65, $p < 0.05$	1.16, NS
NEB	2.75, NS	13.19, $p < 0.005$	1.48, NS
Rears	2.32, NS	0.20, NS	2.08, NS
Head dips	0.23, NS	47.25, $p < 0.001$	0.95, NS
% p dips	0.02, NS	0.71, NS	0.09, NS
SAP	0.25, NS	21.34, $p < 0.001$	0.58, NS
% p SAP	1.75, NS	9.47, $p < 0.005$	1.10, NS
Closed arm returns	0.24, NS	0.69, NS	0.34, NS

NEB = nonexploratory behaviour; SAP = stretched attend postures; % p = percent protected. See Table 1 for complementary information.

time-dependent changes over the course of the 5-min test period: with 4 and 496 degrees of freedom, total entries ($F = 6.37$, $p < 0.001$), open entries ($F = 15.99$, $p < 0.001$), closed-entries ($F = 22.55$, $p < 0.001$), percent open entries ($F = 45.63$, $p < 0.001$), percent open time ($F = 12.08$, $p < 0.001$), percent centre time ($F = 35.50$, $p < 0.001$), percent closed time ($F = 69.34$, $p < 0.001$), nonexploratory behaviour ($F = 13.56$, $p < 0.001$), rears ($F = 29.20$, $p < 0.001$), head-dips ($F = 29.92$, $p < 0.001$), SAPs ($F = 32.33$, $p < 0.001$), closed arm returns ($F = 3.90$, $p < 0.004$).

During the first minute of the test, behaviour was characterized by a high (50%) percentage of time spent on the central platform, with approximately equal (25% each) time spent in open and closed arms; entries into open and closed arms were also equivalent at this time. Rearing, nonexploratory behaviour, and closed arm returns were low during the first minute, and stretched attend postures and head-dipping occurred with relatively high frequencies. By the second minute, total entries, rearing, closed entries, closed time, and closed arm returns had increased markedly and generally remained at these higher levels until the end of the test. Nonexploratory behaviour, principally grooming, showed a linear increase from the first to fifth min. In contrast, open-arm entries, percent open-arm entries, percent open-arm time, percent central platform time, head-dips and stretched attend postures showed linear decreases over the test session. Thus, relative to the first min, the final min of the test was characterized by profound open-arm avoidance, low levels of risk assessment and high levels of closed-arm activities (entries, time, returns, rears, and non-

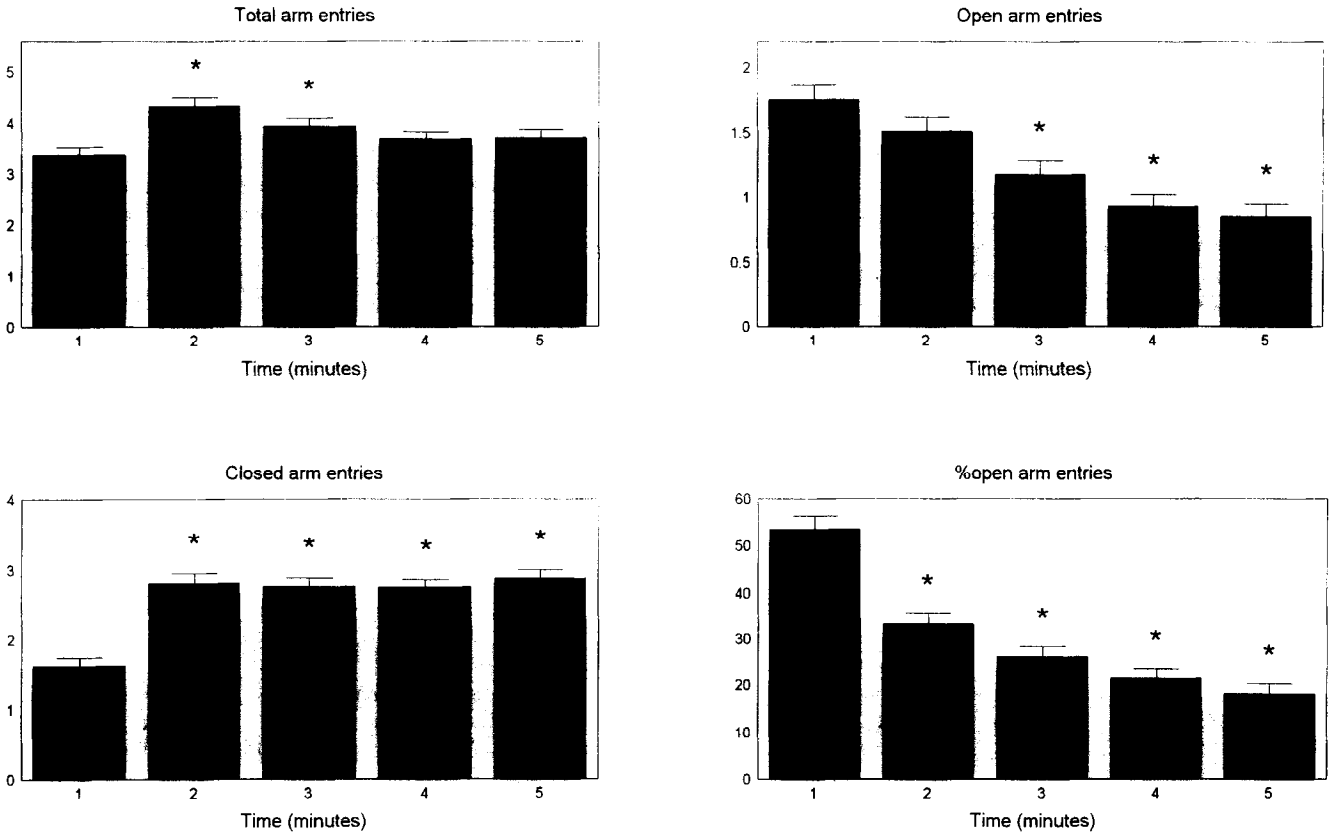

FIG. 1. Minute-by-minute changes in total arm entries, open entries, closed entries, and percent open entries in 125 control DBA/2 mice tested for 5 min in the elevated plus-maze. *$p < 0.01$ vs. min 1.

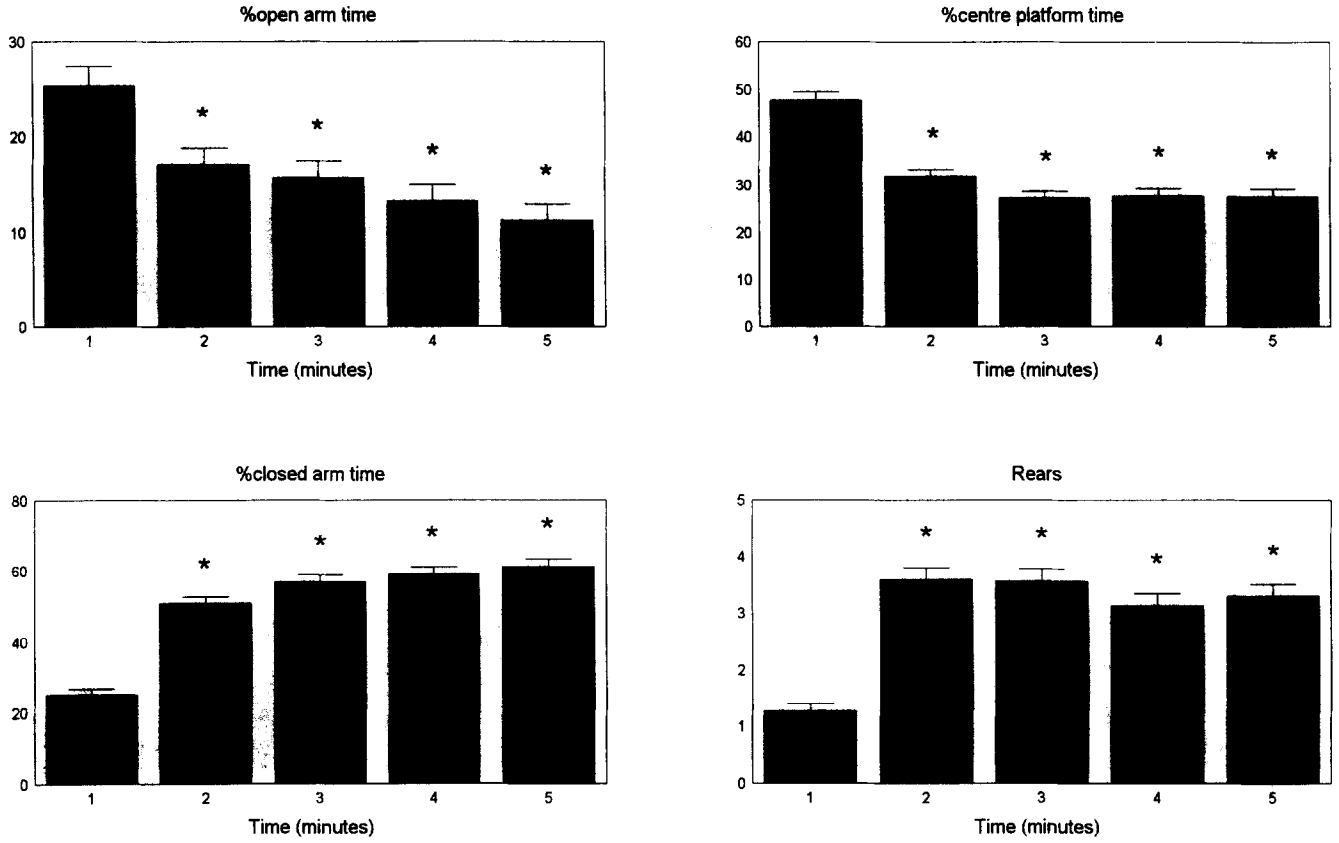

FIG. 2. Minute-by-minute changes in percent open time, percent centre time, percent closed times and rearing in 125 control DBA/2 mice tested for 5 min in the elevated plus-maze. *$p < 0.01$ vs. min 1.

exploratory behaviour). Overall, these data are consistent with high levels of risk assessment (information-gathering) from the centre platform during the first min of the test period, coupled with a lack of discrimination between open and closed arms. However, from the second min onwards, mice displayed a marked preference for the closed arms, with reciprocal reductions in centre platform activities and open-arm exploration.

Experiment 3

5-min control profile. Table 3 summarizes the test/retest behavioural profile and corresponding ANOVA statistics for mice exposed to the plus maze for 5 min (Trial 1), injected with saline, and retested for 5 min 24 h later (Trial 2). Results showed that Trial 2 was associated with significant increases in total entries, closed entries, and closed time, together with significant reductions in centre time, head-dipping and entry latencies. Although percent open time was reduced by 10 points, this change just failed to reach significance.

Trial 1 equivalence (2-min exposure). Single-factor ANOVAs were used to assess the behavioural comparability of groups prior to drug administration (i.e., Trial 1; see Table 4). With 2 and 27 degrees of freedom and an $F_{crit0.05}$ of 3.35, the following F-values were obtained: total entries ($F = 0.88$), open entries ($F = 0.68$), closed entries ($F = 2.30$), percent open entries ($F = 4.03$, $p < 0.05$), percent open time ($F = 0.64$), percent centre time ($F = 0.79$), percent closed time ($F = 1.24$), entry latency ($F = 1.47$), nonexploratory behaviour ($F = 2.20$), rears ($F = 0.17$), head-dips ($F = 0.58$), percent protected head-dips ($F = 0.35$), stretched attend postures ($F = 0.03$), percent protected stretched attend postures ($F = 2.16$), and closed-arm returns ($F = 1.05$). With the exception of percent open-arm entries (which was significantly higher than control in the '1.0 mg/kg' condition, $p < 0.05$), these analyses confirm that groups were behaviourally equivalent prior to drug treatment.

Effects of retesting and posttrial scopolamine. Data and ANOVA statistics are summarized in Tables 4 and 5. Even with the reduced test duration (2-min test and retest), retesting on the maze resulted in significant overall reductions in open-arm entries, percent open-arm entries, percent open-arm time, percent centre time, and head-dips. In contrast, increases were observed for closed-arm entries, percent closed time, closed returns, and rearing. No 'trials' effects were observed for total arm entries, percent protected head-dips, stretched attend postures, percent protected stretched attend postures, entry latency, or nonexploratory behaviour. Only one behavioural measure (percent open entries) showed a significant drug × trial interaction. Further analysis indicated that this was due to the abnormally high Trial 1 score (52.6 ± 6.4%) for the 1.0 mg/kg group. Thus, posttraining scopolamine, again, failed to influence behaviour on retest.

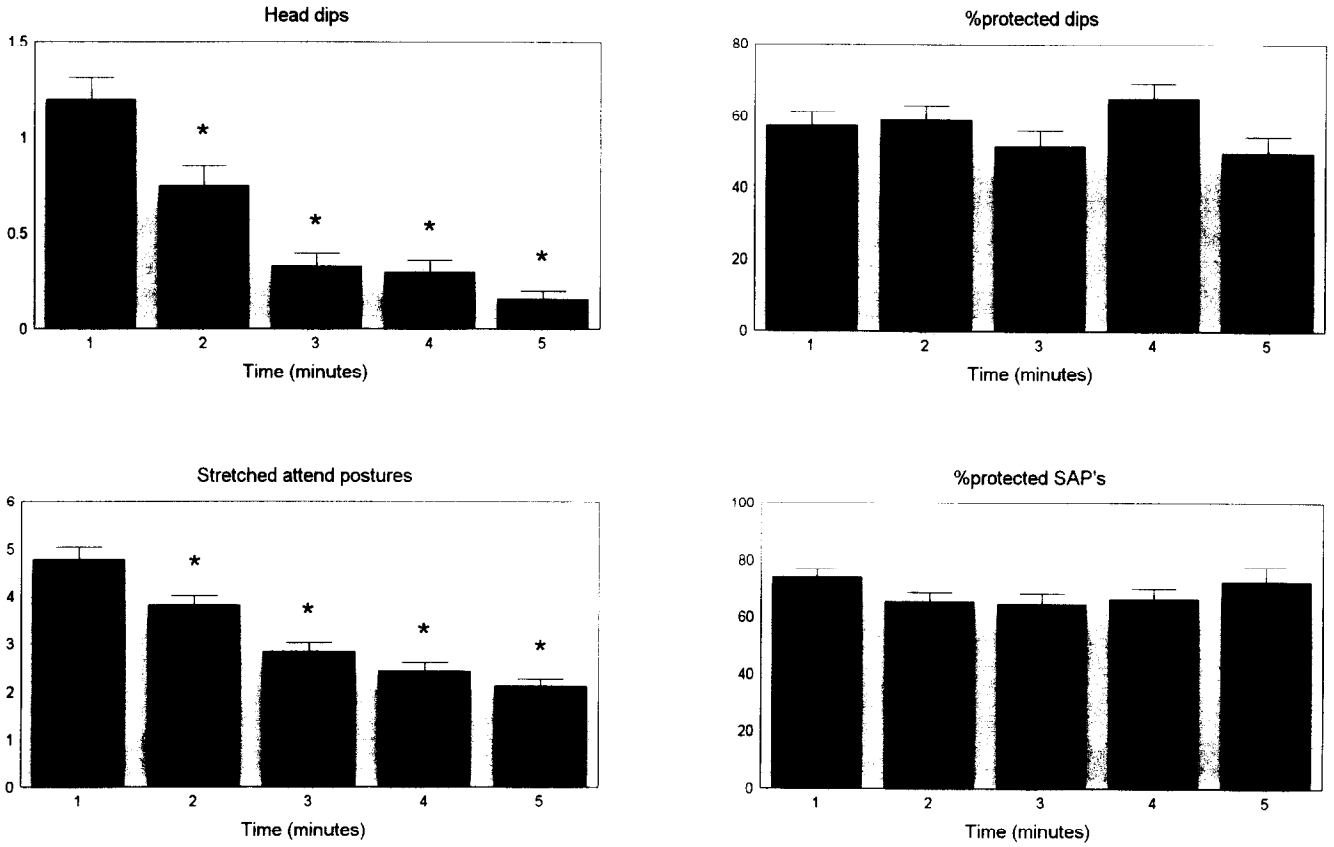

FIG. 3. Minute-by-minute changes in head dips, percent protected head dips, stretched attend postures, and percent protected stretched attend postures in 125 control DBA/2 mice tested for 5 min in the elevated plus-maze. *$p < 0.01$ vs. min 1.

Effects of trial 1 duration on trial 2 profiles. Results are summarized in Table 6. ANOVA showed that the duration of Trial 1 (2-min vs. 5-min) had very little effect upon behavioural patterns observed during a conventional Trial 2. Indeed, the only differences were that mice initially exposed for 2 min had higher entry latencies and higher percent centre time scores than those exposed for a full 5 min. These data suggest that the first few minutes of Trial 1 are of critical importance to the acquisition of information that subsequently determines exploratory patterns on Trial 2.

DISCUSSION

In agreement with previous results from this laboratory (21,34,35), and elsewhere (1,4,16,38,41), present findings indicate that reexposure to the elevated plus-maze test results in

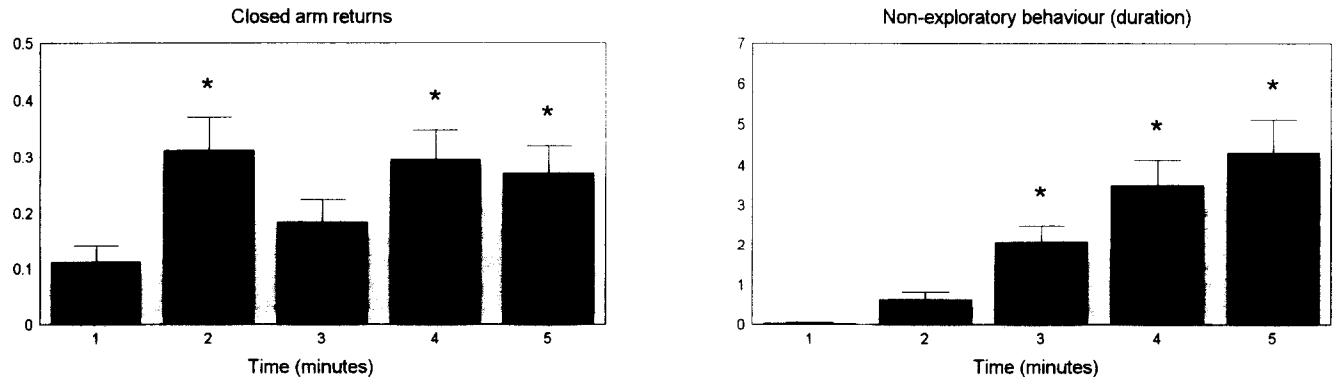

FIG. 4. Minute-by-minute changes in closed-arm returns and nonexploratory behaviour in 125 control DBA/2 mice tested for 5 min in the elevated plus-maze. *$p < 0.01$ vs. min 1.

TABLE 3

TEST/RETEST BEHAVIOURAL (EXPERIMENT 3) PROFILE OF MALE DBA/2 MICE EXPOSED FOR 5-min TO THE MAZE (TRIAL 1), INJECTED WITH SALINE, AND REEXPOSED 24 h LATER (TRIAL 2)

Behaviour	Trial 1	Trial 2	F (1,9)
Total entries	18.2 ± 1.2	24.1 ± 2.5	6.27, $p < 0.05$
Open entries	5.9 ± 0.4	5.8 ± 1.1	0.05, NS
Closed entries	12.3 ± 1.1	18.3 ± 2.0	10.80, $p < 0.01$
Percent open entries	32.9 ± 2.8	22.8 ± 4.0	4.22, NS
Percent open time	12.6 ± 1.3	12.7 ± 2.2	0.05, NS
Percent centre time	29.9 ± 1.3	21.5 ± 1.9	31.69, $p < 0.001$
Percent closed time	57.5 ± 1.6	65.8 ± 2.9	8.20, $p < 0.025$
Entry latency	13.8 ± 2.1	1.2 ± 0.3	35.69, $p < 0.001$
NEB	14.1 ± 3.6	19.0 ± 5.5	0.50, NS
Rears	17.6 ± 1.5	20.8 ± 1.3	2.90, NS
Head dips	13.8 ± 1.6	4.8 ± 1.3	23.98, $p < 0.001$
% p Dips	62.2 ± 6.1	64.4 ± 10.3	0.03, NS
SAP	13.5 ± 1.6	14.6 ± 1.9	0.36, NS
% p SAP	57.9 ± 6.1	54.2 ± 7.6	0.13, NS
Closed arm returns	0.6 ± 0.3	1.2 ± 0.3	1.45, NS

NEB = nonexploratory behaviour; SAP = stretched attend postures; % p = percent protected.

significant behavioural changes in rodents. Also consistent with previous work (34,35), the strength of these between-trial changes varies across experiments. Thus, more profound test-retest differences were noted in Experiment 1 than in the 5-min control condition in Experiment 3. Why this variability should exist is not immediately apparent, especially in view of the rather similar control profiles in the two studies. Nevertheless, the present ethological analysis demonstrates that changes occur in many behaviours and not just the conventional plus-maze indices. Measures that displayed consistent between-trial alterations in the 5-min test/retest paradigm are entry latency (decrease), centre time (decrease), head-dipping (decrease), percent open entries (decrease), closed-arm entries (increase), and closed time (increase). Thus, on retest, mice move more rapidly from the centre platform into a closed arm, spend more time in the closed arms and less time on the centre platform, and show less exploratory head-dipping. Recently, Dawson and colleagues (4) have argued that the apparent loss of benzodiazepine efficacy in maze-experienced animals (7,8,9, 22,23,34,35) may be an artefact related to between-trials habituation of locomotor activity. However, present data seem to be at variance with this proposal. In particular, the results of Experiments 1 and 3 show that total entry scores do not differ between trials, and closed-arm entries (a more valid index of locomotor activity (3,33) actually show an increase.

The pattern of behavioural change seen between trials is consistent with the retention of spatial information over the 24-h test-retest interval (9,35). In this context, the profile seen in the 2-min control group in Experiment 3 indicates that the first few minutes of Trial 1 are crucial to this learning experience. Indeed, if anything, the behavioural changes observed in these animals were even more convincing than those seen in the 5-min control group within the same study. Also consistent with this notion of rapid acquistion is the finding that the duration of Trial 1 (2 vs. 5 min) has very little influence on 5-min retest profiles. In other words, an additional 3-min maze experience on Trial 1 does not substantively alter behaviour on 24-h retest. This conclusion is supported by the results of the min-by-min analysis of behaviour during initial maze exposure (Experiment 2). This study, conducted on our computer database containing profiles of 125 control mice, confirmed that behaviour during the early stages of Trial 1 (in particular, the first minute) differs markedly from that observed in the remainder of the session. High levels of risk assessment (information-gathering in the form of stretched attend postures; head-dipping) from the central platform were initially observed, with little discrimination evident between open and closed arms. However, by the second minute, mice had already begun to evidence a preference for the closed arms over both the centre platform and open arms, a preference

TABLE 4

EFFECTS OF SCOPOLAMINE (0.1-1.0 mg/kg, IP), ADMINISTERED IMMEDIATELY AFTER TRIAL 1 (2-min), ON THE 24 h RETEST (2-min) BEHAVIOURAL PROFILE OF MALE DBA/2 MICE

Scopolamine HBr

Behaviour	Saline/T1	Saline/T2	0.1 mg/kg/T1	0.1 mg/kg/T2	1.0 mg/kg/T1	1.0 mg/kg/T2
Total entries	8.5 ± 1.4	8.7 ± 1.1	8.8 ± 1.4	9.9 ± 1.2	6.7 ± 0.6	8.9 ± 1.8
Open entries	2.5 ± 0.4	2.0 ± 0.5	2.9 ± 0.8	1.6 ± 0.3	3.5 ± 0.5	1.9 ± 0.6
Closed entries	6.0 ± 1.4	6.7 ± 0.8	5.9 ± 1.1	8.3 ± 1.1	3.2 ± 0.5	7.0 ± 1.2
% open entries	34.5 ± 5.8	20.6 ± 4.6	28.3 ± 6.6	16.0 ± 4.1	52.6 ± 6.4	15.4 ± 3.8
% open time	14.3 ± 4.8	9.7 ± 2.8	9.9 ± 3.3	6.4 ± 1.9	13.9 ± 4.3	7.7 ± 2.4
% centre time	37.7 ± 2.4	31.3 ± 2.9	36.2 ± 2.2	27.6 ± 2.6	42.1 ± 5.0	32.9 ± 3.2
% closed time	48.0 ± 4.9	59.0 ± 4.0	53.9 ± 3.2	66.0 ± 3.4	44.0 ± 5.1	59.4 ± 7.3
Entry latency	15.8 ± 3.1	3.2 ± 0.7	10.6 ± 1.4	2.9 ± 0.9	15.6 ± 2.5	15.1 ± 13.4
NEB	0.9 ± 0.6	4.1 ± 2.6	3.1 ± 1.0	6.4 ± 2.5	6.7 ± 2.7	5.6 ± 2.5
Rears	6.8 ± 1.2	11.1 ± 1.5	7.8 ± 1.0	7.8 ± 1.2	7.5 ± 1.5	9.3 ± 1.7
Head dips	7.9 ± 1.1	3.4 ± 1.1	7.9 ± 1.1	2.9 ± 0.7	9.5 ± 1.4	2.3 ± 0.5
% p Dips	55.8 ± 10.7	58.9 ± 11.3	63.0 ± 8.5	46.0 ± 14.1	51.1 ± 11.2	57.5 ± 13.2
SAP	5.9 ± 1.0	7.0 ± 1.1	6.0 ± 0.5	6.6 ± 1.0	6.0 ± 1.1	7.5 ± 1.1
% p SAP	69.7 ± 11.3	66.0 ± 9.4	80.0 ± 5.5	74.2 ± 9.0	54.4 ± 9.0	66.5 ± 10.6
Closed returns	0.4 ± 0.2	0.8 ± 0.4	0.4 ± 0.2	1.4 ± 0.3	0.1 ± 0.1	0.4 ± 0.2

See text and Table 5 for associated inferential statistics. T1 = trial 1; T2 = trial 2; NEB = nonexploratory behaviour; SAP = stretched attend postures; % p = percent protected.

accompanied by high levels of closed-arm activities such as rearing, grooming and returns. This profile would be consistent with rapid spatial learning, followed by avoidance of the potentially dangerous sections of the maze. It would, therefore, seem logical to propose that this within-session learning transfers across trials, resulting in the retest behavioural changes reported above. Although limited to one behavioural measure only (% time open), it has indeed been reported that rats begin Trial 2 in the 'zero-maze' at a response level not dissimilar to that seen at the end of Trial 1, but markedly lower than observed at the beginning of Trial 1 (38). Whether or not such learning represents a phobia acquisition, as suggested by File and Zangrossi (10), remains to be determined. Nevertheless, our data are consistent with an experientially induced sensitization of fear reactions to the plus-maze (41).

The anticholinergic compound, scopolamine, is well-known for its ability to produce retention deficits in animals and humans (e.g., 2). Although it has been argued that pretraining administration produces more profound 'amnestic' effects (36), immediate posttraining administration has also been found to effectively disrupt retention in a range of tasks, including those involving spatial memory (13,14,20,26,27,29, 40). A posttraining paradigm was employed in the present studies as: 1. scopolamine pretreatment has been shown to radically disrupt behaviour in the plus-maze (5,6,31,38), and 2. major interpretative difficulties are associated with pretrial administration (15,24). The results obtained in Experiment 1 show that posttrial scopolamine (0.1–1.0 mg/kg) is ineffective in altering the plus-maze retest profile in a normal 5-min test/retest protocol (i.e., did not impair retention of information from Trial 1). Cholinergic blockade was also ineffective in Experiment 3, in which the duration of Trial 1 was shortened to 2 min. Although the data reported for this analysis (Tables 4 and 5) naturally compared Trial 1 with the first 2 min of Trial 2, further between-group analyses for the full 5-min of Trial 2 (data not shown) failed to reveal any significant effects of posttraining scopolamine.

Although it might be argued that the dose-range currently used was not high enough, it should be noted that the mouse strain employed is very sensitive to the behavioural effects of scopolamine (31) and that the compound is effective in other memory tasks in doses as low as 0.4–1.0 mg/kg (26,29). Nevertheless, positive effects have been previously reported for scopolamine in a version of the plus-maze that was developed specifically for the study of learning and memory (18). In this test, mice are given a single forced exposure to an open arm of the maze and the latency to move to a closed arm is recorded (transfer latency); on 24-h retest, transfer latencies are found to be significantly reduced. Although pretrial systemic scopolamine partially prevented the reduction in transfer latencies on retest, this effect was observed at one dose only (3 mg/kg, but not 1 or 6 mg/kg), and the drug was seen to enhance transfer latencies during Trial 1 (i.e., at 'acquisition') (19). However, because the peripherally acting compound, butylscopolamine, also prolonged transfer latencies on Trial 1 but did not impair retention, the authors concluded that scopolamine had produced a specific impairment of memory for the aversive open arms. In a related study, the same research group has also shown that posttrial intraventricular administration of scopolamine (10–20 μg) partially reverses the between-trials reduction in transfer latencies (18). More recently, Miyazaki et al. (25) have shown that systemic scopolamine (0.5 and 1.0 mg/kg), adminstered prior to Trial 1, partially prevents the between-trials reduction in transfer latencies without affecting performance on Trial 1. Thus, despite our reservations about the behaviourally disruptive effects of scopolamine (5,6,31,38), and the precise relevance of the single-measure 'transfer latency' paradigm to the ethological plus-maze protocol (e.g., 'forced' exposure to open arms vs. 'free' exploration of the maze), additional studies using pretrial scopolamine administration may be warranted.

In conclusion, present results confirm that retesting on the elevated plus-maze is associated with quite marked behavioural changes indicative of aversive learning. This learning,

TABLE 5

ANOVA SUMMARY STATISTICS FOR EXPERIMENT 3

Behaviour	Drug (df 2,27)	Trials (df 1,27)	Interaction (df 2,27)
Total entries	0.63, NS	1.42, NS	0.35, NS
Open entries	0.32, NS	10.51, $p < 0.005$	0.88, NS
Closed entries	1.58, NS	8.93, $p < 0.01$	1.36, NS
% open entries	1.77, NS	39.12, $p < 0.001$	5.67, $p < 0.01$
% open time	0.45, NS	6.80, $p < 0.025$	0.18, NS
% centre time	0.40, NS	17.58, $p < 0.001$	2.11, NS
% closed time	1.03, NS	23.07, $p < 0.001$	0.25, NS
Entry latency	1.27, NS	1.99, NS	0.51, NS
NEB	1.15, NS	1.46, NS	0.95, NS
Rears	0.29, NS	4.41, $p < 0.05$	1.66, NS
Head dips	0.14, NS	38.26, $p < 0.001$	0.85, NS
% p Dips	0.04, NS	0.09, NS	0.73, NS
SAP	0.16, NS	1.38, NS	0.08, NS
% p SAP	1.32, NS	0.02, NS	0.70, NS
Closed arm returns	3.39, $p < 0.05$	6.76, $p < 0.025$	1.01, NS

NEB = nonexploratory behaviour; SAP = stretched attend postures; % p = percent protected. See Table 4 for complementary information.

TABLE 6

INFLUENCE OF TRIAL 1 DURATION (2 *vs.* 5 min) ON TRIAL 2 (5-min) PROFILES IN MALE DBA/2 MICE

Behaviour	5-min Trial 1	2-min Trial 1	F(1,18)
Total entries	24.1 ± 2.5	19.8 ± 2.0	1.78, NS
Open entries	5.8 ± 1.1	4.1 ± 1.0	1.31, NS
Closed entries	18.3 ± 2.0	16.7 ± 1.2	0.48, NS
Percent open entries	22.8 ± 4.0	19.0 ± 4.2	0.43, NS
Percent open time	12.7 ± 2.2	10.3 ± 2.3	0.56, NS
Percent centre time	21.5 ± 1.9	28.7 ± 2.4	5.62, $p < 0.05$
Percent closed time	65.8 ± 3.0	61.0 ± 3.1	1.25, NS
Entry latency	1.2 ± 0.3	3.2 ± 0.7	6.52, $p < 0.025$
NEB	19.0 ± 5.5	15.5 ± 4.4	0.25, NS
Rears	20.8 ± 1.3	23.2 ± 2.0	1.00, NS
Head dips	4.8 ± 1.2	7.2 ± 2.0	1.01, NS
% p Dips	64.4 ± 10.3	52.9 ± 11.3	0.57, NS
SAP	14.6 ± 1.9	14.2 ± 1.7	0.03, NS
% p SAP	54.2 ± 7.6	63.0 ± 8.0	0.63, NS
Closed arm returns	1.2 ± 0.3	2.0 ± 0.6	1.53, NS

NEB = nonexploratory behaviour; SAP = stretched attend posture; % p = percent protected.

associated with increased open-arm avoidance, is crucially dependent upon information acquired during the initial stages of Trial 1. Thus, similar changes are observed when Trial 1 is fore-shortened to 2 min, behaviour on Trial 2 is largely independent of the duration of Trial 1 (2 vs. 5 min), and response patterns on the maze differ markedly following the first minute of Trial 1. Although it remains possible that higher doses of scopolamine or pretrial administration of the compound may have been effective, present data provide little evidence that cholinergic mechanisms are involved in the sensitization of fear that appears to occur between initial and subsequent exposures to the elevated plus-maze. Further work is clearly required to characterize the substrates involved.

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