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Behavioural Effects in Mice of Subchronic Chlordiazepoxide, Maprotiline and Fluvoxamine. II. The Elevated Plus-Maze

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RODGERS, R. J., M. G. CUTLER AND J. E. JACKSON. *Behavioural effects in mice of subchronic chlordiazepoxide, maprotiline and fluvoxamine. II. The elevated plus-maze.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 127–136, 1997.—In view of apparent commonalities in the aetiology, symptomatology, and pharmacotherapy of anxiety and depressive disorders, the present study compares the effects of the benzodiazepine, chlordiazepoxide (1.0–8.0 mg/kg), the selective noradrenaline (NA) reuptake inhibitor, maprotiline (0.5–10.0 mg/kg), and the serotonin (5-HT)-selective reuptake inhibitor, fluvoxamine (2.0–8.0 mg/kg), on the behaviour of mice in the elevated plus-maze test of anxiety. To more accurately reflect the clinical situation, subjects were treated daily for 21 days prior to testing, and comprehensive behavioural profiles were obtained through the application of an ethological scoring technique. Results show that subchronic treatment with chlordiazepoxide produced clear anxiolytic-like effects at the highest dose tested, coupled with an inhibition of risk assessment over the entire dose range. With the exception of risk assessment measures, anxiolytic-like effects were also seen with a low dose (0.5 mg/kg) of maprotiline; these effects were lost at higher doses. In contrast to these data, fluvoxamine produced minimal behavioural change under present test conditions. Findings are discussed in relation to the relative efficacy of selective monoamine reuptake inhibitors in the treatment of anxiety disorders, and the nature of anxiety evoked in mice by exposure to the elevated plus-maze. © 1997 Elsevier Science Inc.

Anxiety Plus-maze Anxiolytics Antidepressants Chlordiazepoxide Maprotiline Fluvoxamine Panic Mice

THE RELATIONSHIP between anxiety and depression has been a source of much psychiatric debate during the course of this century (34). Current views emphasize not only the high degree of symptom overlap, comorbidity, and genetic commonality of these disorders (41,49,51,59), but also the absence of a clear therapeutic demarcation (50,70). For example, panic disorder has for many years been successfully treated with tricyclic antidepressants and monoamine oxidase inhibitors (43,51,52,61,66), with similar results now being established for the more serotonin-selective reuptake inhibitors (SSRIs) (1,21,40,50,56,72). Furthermore, recent research has revealed that chronic imipramine treatment can be just as useful as benzodiazepine therapy in the management of patients with generalized anxiety disorder (34,39,60,70).

In this context, it is perhaps surprising that antidepressant drugs have generally been found not to produce anxiolytic-like effects in rodent models of anxiety. Thus, negative findings have been reported for traditional antidepressants in conflict/conditioned supression tasks (26,27,42,46) as well as light/dark exploration (16,17,54,74), potentiated startle 9,33, separation- and shock-induced ultrasonic calling (19,29,71), and elevated plus-maze (7,12,22,45,57) paradigms. However, these animal studies have involved acute drug administration whereas the clinical efficacy of agents like imipramine only gradually emerges over a period of chronic treatment.

That clinical and preclinical experience can coincide in this field is suggested by the enhancement of anxiety seen with acute antidepressant treatment (particularly SSRIs) in panic patients (1,51,72) and in some animal models (2,6,30–32,44,55,58). Furthermore, although negative effects with

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TABLE 1

ANOVA SUMMARY STATISTICS FOR THE EFFECTS OF EXPERIMENTAL MANIPULATIONS* ON PLUS-MAZE BEHAVIOUR IN CD1 MICE

Variable	F	*p*
Total entries	3.19	0.002
Open entries	3.68	0.001
Closed entries	2.56	0.01
Total rears	1.43	n.s.
% Open entries	2.79	0.005
% End-open entries	2.44	0.01
% Open arm time	3.05	0.002
% End-open time	2.48	0.01
% Centre time	1.53	n.s.
% Closed arm time	2.01	0.05
Total head-dips	2.27	0.02
End-open head-dips	4.08	0.0001
Total SAP	4.31	0.0001
End-open SAP	1.97	0.05
Rearing duration (s)	0.92	n.s.
Sniff duration (s)	1.19	n.s.
Grooming duration (s)	2.43	0.02
Flat back approach duration (s)	0.97	n.s.
%p head-dips	2.42	0.02
%p SAP	3.30	0.001
%p sniff	2.28	0.02
%p flat back approach	2.00	0.05

* 21-day handling/injection; 21-day treatment with chlordiazepoxide, maprotiline or fluvoxamine. Degrees of freedom = 10,97; n.s. = non-significant.

TABLE 2

THE EFFECTS OF DAILY HANDLING AND INJECTION (21 DAYS) ON THE BEHAVIOUR OF CD1 MICE IN THE ELEVATED PLUS-MAZE

Variable	Uninjected Control (n = 16)	Saline Control (n = 14)
Total entries	29.8 ± 1.6	25.9 ± 2.2
Open entries	16.2 ± 1.1	14.6 ± 1.8
Closed entries	13.6 ± 1.3	11.3 ± 1.0
Total rears	21.9 ± 2.6	20.5 ± 2.4
% Open entries	55.0 ± 3.5	54.8 ± 3.4
% End-open entries	19.4 ± 1.4	21.5 ± 1.8
% Open arm time	28.8 ± 2.1	26.8 ± 3.4
% End-open time	12.1 ± 1.1	10.5 ± 1.5
% Centre time	36.6 ± 2.1	38.5 ± 2.6
% Closed arm time	34.6 ± 2.6	34.7 ± 2.4
Total head-dips	15.6 ± 1.1	17.0 ± 1.3
End-open head-dips	4.8 ± 0.7	4.0 ± 0.7
Total SAP	14.7 ± 1.3	20.3 ± 1.2*
End-open SAP	3.1 ± 0.3	3.4 ± 0.4
Rearing duration (s)	18.2 ± 2.4	14.3 ± 1.7
Sniff duration (s)	38.2 ± 4.6	31.9 ± 3.4
Grooming duration (s)	13.5 ± 1.8	13.5 ± 3.1
Flat back approach duration (s)	37.3 ± 3.7	25.3 ± 3.8
%p head-dips	41.7 ± 4.2	47.9 ± 5.9
%p SAP	50.9 ± 5.0	60.9 ± 4.0
%p sniff	87.2 ± 2.2	85.7 ± 2.9
%p flat back approach	24.4 ± 3.4	22.1 ± 3.8

Data are presented as mean values ± SEM.
* $p < 0.05$.

chronic antidepressant treatment have been reported in animal studies (9,12,23,58), results using this clinically relevant approach have generally been more encouraging than those obtained in acute studies. For example, anxiolytic-like effects have been seen in a range of animal models following chronic treatment with a number of first-generation (e.g., imipramine, amitriptyline, phenelzine) and second-generation (e.g., mianserin, paroxetine, tianeptine) compounds (4,5,14,24,26–28,30). Data from the elevated plus-maze test have been more variable in that both positive (8,31,38,44,62) and negative (12,23,58) effects have been reported following chronic antidepressant treatment. However, it is noticeable that in the latter studies positive effects have generally been seen with SSRI-antidepressants and negative results with nonselective agents such as imipramine.

In view of this pattern of results, we have recently undertaken a large-scale comparative study of the effects of chronic anxiolytic (chlordiazepoxide, buspirone, ondansetron) and antidepressant (maprotiline, fluvoxamine, tianeptine) treatment on the behaviour of mice in two ethological models that have successfully been used in studies on the behavioural pharmacology of anxiety; the social interaction (28) and elevated plus-maze (65) tests. There are major advantages to the ethopharmacological technique in that it has high ecological validity and provides comprehensive behavioural profiles of drug action, thereby permitting conclusions regarding the behavioural specificity of drug effects within a single test situation. In the present article, we report a direct comparison of the subchronic effects of chlordiazepoxide, maprotiline and fluvoxamine in the murine plus-maze. A companion article (20) presents data from the parallel study using the murine social interaction test, while our findings with buspirone, ondansetron and tianeptine will be reported separately.

METHOD

Animals

Subjects were adult male CD1 mice (Charles River, UK), weighing 23–45g and housed in groups of 10 or 11 (cage size: 45 × 28 × 13 cm) for 3 weeks prior to the experiment. They were maintained under a reversed 12L:12D cycle (lights off 0700 h) in a temperature (21 ± 1°C) and humidity (50 ± 5%) controlled environment. Food and water were freely available. All mice were experimentally naive.

Drugs

Drugs used were chlordiazepoxide hydrochloride (Roche Products, London, UK), fluvoxamine maleate (SmithKline

TABLE 3

EFFECTS OF SUBCHRONIC TREATMENT WITH CHLORDIAZEPOXIDE (1.0-8.0 MG/KG, DAILY 21 DAYS) ON THE BEHAVIOUR OF CD1 MICE IN THE ELEVATED PLUS-MAZE TEST OF ANXIETY

Variable	Saline (n = 14)	1.0 mg/kg (n = 8)	4.0 mg/kg (n = 10)	8.0 mg/kg (n = 8)
Closed entries	11.3 ± 1.0	11.8 ± 1.3	11.1 ± 1.4	12.9 ± 1.5
Total rears	20.5 ± 2.5	24.9 ± 3.2	17.6 ± 3.7	16.7 ± 3.7
Total head-dips	17.0 ± 1.3	17.6 ± 2.6	17.4 ± 2.0	22.2 ± 2.5
End-open SAP	3.4 ± 0.4	2.7 ± 0.5	3.1 ± 0.6	4.1 ± 0.5
% Open entries	54.8 ± 3.4	60.1 ± 3.9	63.6 ± 4.7	68.9 ± 2.6
% End-open entries	21.5 ± 1.8	22.6 ± 2.3	25.8 ± 2.7	27.1 ± 1.4
% End-open time	10.5 ± 1.5	11.1 ± 1.1	15.1 ± 3.3	16.7 ± 1.5
% Centre time	38.5 ± 2.6	31.6 ± 2.6	31.8 ± 3.9	31.1 ± 2.0
% Closed arm time	34.7 ± 2.4	35.2 ± 2.2	33.9 ± 3.6	27.0 ± 3.1
Rearing duration (s)	14.3 ± 1.7	20.7 ± 3.4	11.6 ± 3.0	16.7 ± 4.5
Sniff duration (s)	31.9 ± 3.4	30.3 ± 5.1	30.0 ± 6.1	20.4 ± 4.5
Grooming duration (s)	13.5 ± 3.1	13.5 ± 1.4	16.3 ± 2.9	10.7 ± 2.0
Flat back approach duration (s)	25.3 ± 3.8	32.6 ± 3.9	26.2 ± 4.0	27.1 ± 4.9
%p sniff	85.7 ± 2.9	82.9 ± 4.9	83.4 ± 3.8	76.1 ± 5.4
%p flat back approach	22.1 ± 3.8	23.5 ± 4.9	24.0 ± 3.7	21.8 ± 2.0

Statistical analysis failed to reveal significance for these measures. See Figure 1 for complementary data.

Beecham, London, UK), and maprotiline hydrochloride (Sigma, Poole, UK). All compounds were dissolved in physiological saline and administered intraperitoneally in a volume of 1 ml/300 g. Injections were given once daily for 21 days, with the side of injection alternated to avoid peritoneal irritation. Noninjected controls were included in the design to assess the effect of chronic handling and injection.

Apparatus

The elevated plus-maze was a modification of that validated for NIH mice by Lister (45), and comprised two open arms (30 × 5 cm) and two enclosed arms (30 × 5 × 15 cm) that extended from a common central platform (5 × 5 cm). The configuration formed the shape of a plus-sign, with like arms arranged opposite one another, and the apparatus was elevated 60 cm above floor level on a central pedestal. The maze floor was made of black Plexiglas while the side and end walls of the enclosed arms were made from clear Plexiglas. As reported previously (10–12), grip on the open arms was facilitated by inclusion of a small raised edge (0.25 cm) around their perimeter and open-arm activity was further encouraged by testing under dim red light (4 × 60 W).

Experimental Procedure

Mice were randomly allocated to 1 of 11 treatment conditions (n = 6–16): uninjected control; saline; 1.0, 4.0, or 8.0 mg/kg chlordiazepoxide; 0.5, 2.0, or 10.0 mg/kg maprotiline; or 2.0, 4.0, or 8.0 mg/kg fluvoxamine. Animals were tail-marked for individual recognition, and treated once daily for 21 days prior to testing. Behavioural testing was conducted during the dark period of the light-dark cycle, when mice are normally most active (0930–1230 h). To facilitate habituation, animals were transported to the laboratory from the holding room at least 1 h before testing. At 30 min following the final injection, animals were individually placed onto the central square of the maze facing an open arm. A 5-min test duration was used and, between subjects, the maze was thoroughly cleaned with both damp and dry cloths. Test sessions were recorded by an overhead video camera linked to a monitor and video recorder in an adjacent room.

Behavioural Analysis

Videotapes were scored blind by a highly trained observer using ethological software (Hindsight, developed in this laboratory by Scott Weiss). Using separate location and behaviour keys, this software permits the real-time scoring of acts and postures by direct keyboard entry to a PC. Data can then be collated by treatment condition and downloaded for statistical treatment. Both conventional and ethological parameters were recorded (65).

The conventional measures comprised: number of open and closed arm entries (arm entry defined as all four paws entering an arm), and time spent on different sections of the maze (including the central platform). The distribution of behaviour on the maze was calculated as "percent total" for frequency (percent open entries: open/total × 100) and duration (percent time spent in open, centre, and closed sections: open time/300 × 100). As pilot studies had indicated that CD1 mice show high levels of open arm entries compared with other strains (e.g., DBA/2, T1, and BALB/c (10)), entries into, and time spent on, the ends of the open arms (defined as distal half of open arm) were also recorded; from these values, percent scores for end-open entries (end-open entries/total × 100) and end-open time (end-open time/300 × 100) were derived.

In addition to the conventional measures, a range of behav-

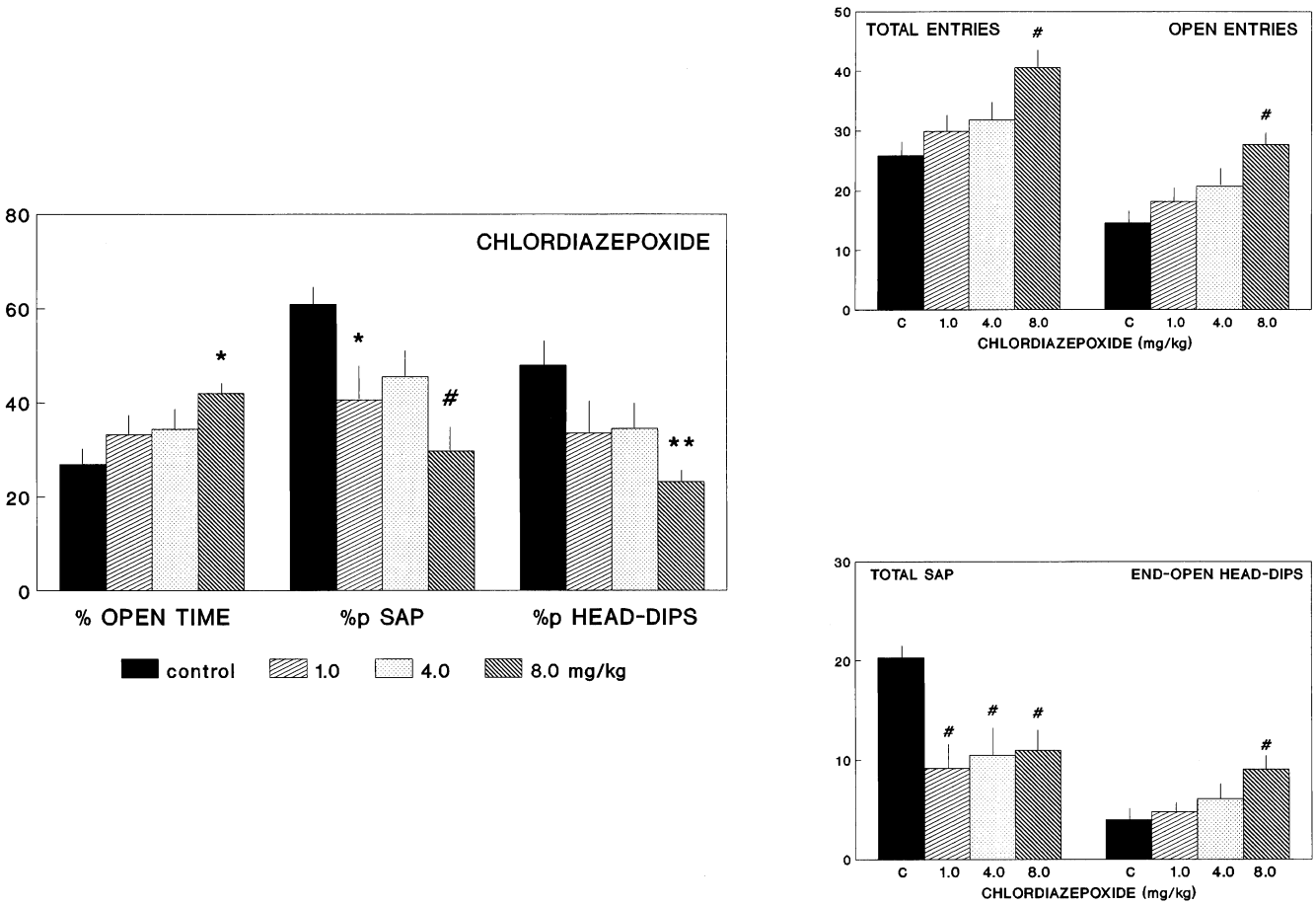

FIG. 1. The effects of subchronic chlordiazepoxide (1.0–8.0 mg/kg; daily for 21 days) on behaviours displayed by male CD1 mice in the elevated plus-maze test of anxiety. Data are presented as mean values ± SEM. See Table 3 for complementary data. %p = percent protected; SAP = stretched attend postures. *$p < 0.05$; **$p < 0.025$; ***$p < 0.01$; #$p < 0.005$ vs. saline control.

iours (encompassing elements of the murine defensive repertoire) were recorded 65. These ethologically derived acts and postures comprised rearing frequency and duration (all rearing occurred against the walls of the closed arms, i.e., supported rearing); the frequency of discrete behaviours such a head-dipping (exploratory movement of head/shoulders over the sides of the maze) and stretched attend postures (SAP; an exploratory posture in which the mouse stretches forward and retracts to original position without locomoting forward); and the duration (s) of prolonged behaviours such as sniffing (olfactory exploration of maze floor and walls with occasional air-sampling), grooming (species-typical sequence beginning with snout, progressing to ears, and ending with whole-body groom), and flat-back approach behaviour (exploratory locomotion where the animal stretches to its full length and cautiously moves forward). It should be noted that control levels of closed arm returns, a measure normally recorded in studies with DBA/2 mice in our laboratory (10–12), were extremely low in CD1 mice and were not therefore scored.

As thigmotactic cues play an important role in plus-maze exploration (68), head-dipping, stretched attend, sniffing, and flat back approach were further differentiated as a function of whereabouts on the maze they were performed. The closed arms and central platform were together designated protected areas of the maze (i.e., offering relative security), while the open arms were designated unprotected areas. Data for the above behavioural elements are therefore given as percent protected scores (%p; protected/total × 100) as well as behaviour totals. Finally, to complement the measurement of end-open entries and end-open time, the frequencies of end-open head-dips and end-open SAP were also recorded.

Statistical Analysis

Data were analysed by single factor (drug treatment) or two-factor (drug treatment × location; repeated measures on location) analyses of variance (ANOVA). Where indicated by significant/near significant F-values, further tests (Dunnett's t-statistic) were performed using the appropriate error variance terms from the ANOVA summary tables.

RESULTS

Table 1 summarizes the main ANOVA statistics, and reveals significant treatment effects on a wide range of behavioural measures. For clarity, the results of follow-up analyses are considered under a number of subheadings.

Control Profiles: Effect of Handling and Injection

Table 2 compares the behavioural profiles of uninjected and saline-injected control animals. In comparison with previ-

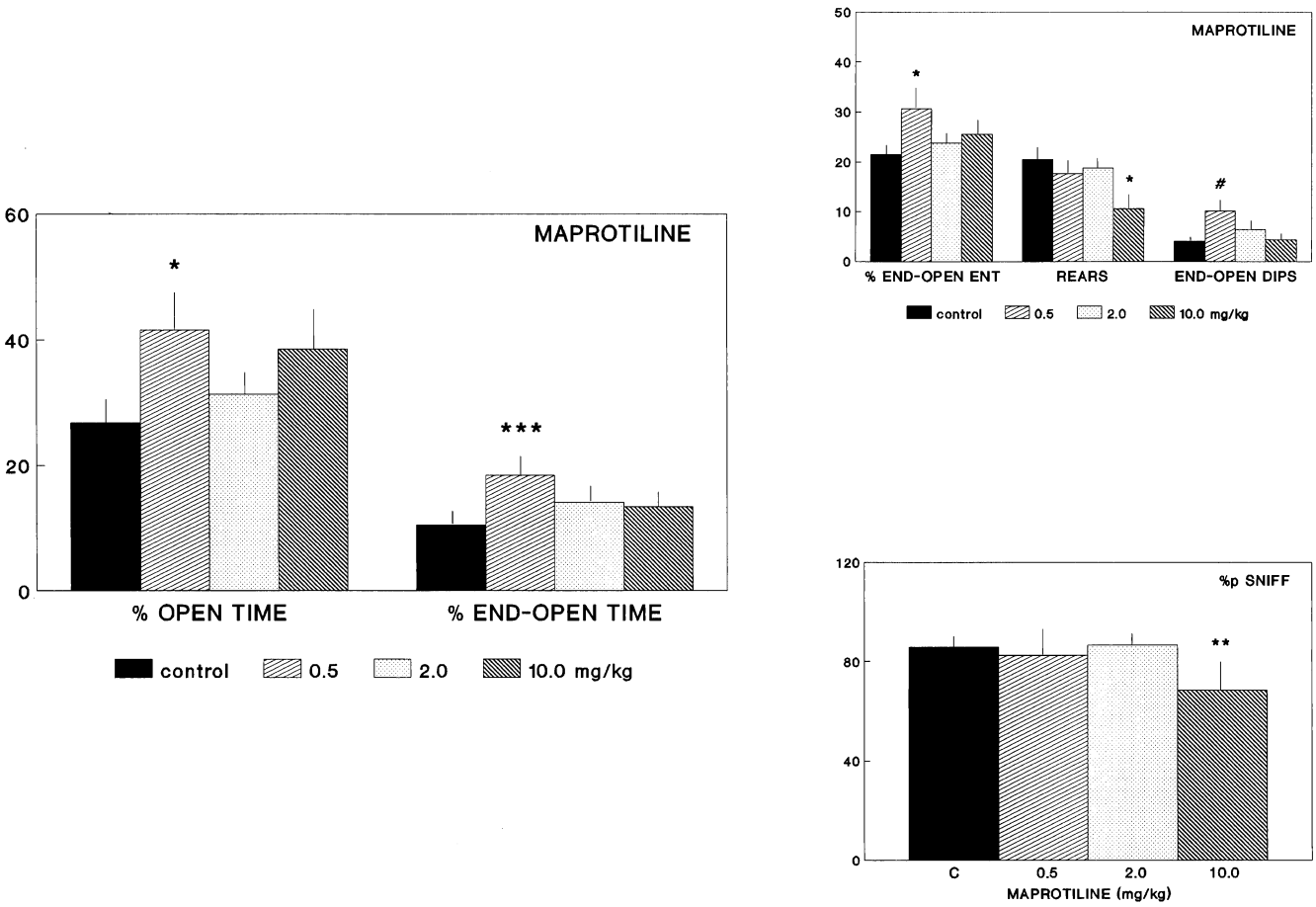

FIG. 2. The effects of subchronic maprotiline (0.5–10.0 mg/kg, daily for 21 days) on behaviours displayed by male CD1 mice in the elevated plus-maze test of anxiety. Data are presented as mean values ± SEM. See Table 4 for complementary data. %p = percent protected. *$p <$ 0.05; **$p <$ 0.025; ***$p <$ 0.01; #$p <$ 0.005 vs. saline control.

ous studies using male DBA/2, T1, and BALB/c mice (10), male CD1 mice generally showed higher levels of plus-maze activity and exploration and, on both conventional (% open entries and time) and ethological indices (e.g., total SAP and percent protected SAP), displayed a lower baseline anxiety profile. Daily handling and injection for 21 days had mimimal effects on plus-maze behaviour, with the only change observed being an increase in total SAP ($p < 0.05$).

Effects of Chlordiazepoxide

The effects of subchronic treatment with chlordiazepoxide are summarized in Figure 1 and Table 3. Behavioural changes were observed primarily at the highest dose tested (8 mg/kg). Significant increases in total entries, open entries, percent open time, and end-open head-dipping were evident at this dose (Fig. 2), while apparent increases in percent open entries and percent end-open time just failed to reach significance (Table 1). Chlordiazepoxide treatment also significantly reduced total SAP across the dose range and percent protected SAP at low and high doses, while percent protected head-dipping was reduced at the high dose only. A two-factor ANOVA on percent time data (treatment × maze location; location = open arms, centre platform, closed arms) revealed a significant treatment by location interaction ($F(20,194) = 2,35$, $p < 0.002$). Follow-up tests showed that the control profile of closed = centre > open ($p < 0.05$) was altered by chlordiazepoxide, with 1.0–4.0 mg/kg resulting in an abolition of spatial preference, and 8.0 mg/kg producing an apparent preference for open arms (42%) over both the centre platform (31%; $p < 0.05$) and closed arms (27%; $p < 0.01$).

Effects of Maprotiline

The effects of maprotiline treatment on plus-maze behaviour are summarized in Figure 2 and Table 4. Most behavioural changes were observed at the lowest dose tested (0.5 mg/kg), and consisted of significant increases in percent end-open entries, percent open time, percent end-open time and end-open head-dipping (Fig. 2). An increase in percent open entries and decrease in percent protected head-dipping closely approached significance (Table 4). No effects were observed at 2.0 mg/kg, while 10 mg/kg produced significant reductions in rearing frequency and percent protected sniffing. Follow-up tests on the significant two-factor ANOVA (details above) indicated that the lowest dose of maprotiline (0.5 mg/kg) altered the control spatial perference (closed = centre > open) such that open arms (41%) became preferred over both the centre platform (31%; $p < 0.05$) and closed arms (28%; $p <$ 0.01). The profile for the 2.0 mg/kg condition did not differ

TABLE 4

EFFECTS OF SUBCHRONIC TREATMENT WITH MAPROTILINE (0.5-10.0 MG/KG, DAILY 21 DAYS) ON THE BEHAVIOUR OF CD1 MICE IN THE ELEVATED PLUS-MAZE TEST OF ANXIETY

Variable	Saline (n = 14)	0.5 mg/kg (n = 9)	2.0 mg/kg (n = 6)	10.0 mg/kg (n = 9)
Total entries	25.9 ± 2.2	29.9 ± 2.7	30.8 ± 2.7	21.8 ± 2.0
Open entries	14.6 ± 1.8	20.8 ± 2.8	18.7 ± 1.9	13.4 ± 1.8
Closed entries	11.3 ± 1.0	9.1 ± 1.0	12.2 ± 1.2	8.4 ± 1.3
% Open entries	54.8 ± 3.4	68.1 ± 3.6	60.2 ± 2.7	61.1 ± 5.3
% Centre time	38.5 ± 2.6	30.6 ± 3.3	37.5 ± 3.1	33.4 ± 4.0
% Closed arm time	34.7 ± 2.4	27.9 ± 3.3	31.1 ± 1.7	27.1 ± 3.7
Total head-dips	17.0 ± 1.3	22.6 ± 2.3	21.0 ± 1.7	15.3 ± 2.1
Total SAP	20.3 ± 1.2	17.7 ± 2.5	20.7 ± 1.4	17.9 ± 1.6
End-open SAP	3.4 ± 0.4	4.6 ± 0.7	4.8 ± 1.0	3.9 ± 0.5
Rearing duration (s)	14.3 ± 1.7	15.2 ± 3.3	14.7 ± 4.1	10.0 ± 3.2
Sniff duration (s)	31.9 ± 3.4	24.7 ± 3.3	26.4 ± 4.5	35.0 ± 5.1
Grooming duration (s)	13.5 ± 3.1	9.6 ± 2.4	5.9 ± 1.1	8.4 ± 2.2
Flat back approach duration (s)	25.3 ± 3.8	48.3 ± 24.7	23.1 ± 3.8	32.7 ± 3.1
%p head-dips	47.9 ± 5.9	29.3 ± 6.3	46.2 ± 7.7	39.1 ± 6.8
%p SAP	60.9 ± 4.0	47.4 ± 3.9	51.7 ± 5.2	49.1 ± 5.7
%p flat back approach	22.1 ± 3.8	23.6 ± 4.8	18.3 ± 4.9	28.2 ± 4.8

Statistical analysis failed to reveal significance for these measures. The low sample size for the 2.0 mg/kg condition was due to data loss resulting from poor quality image on part of a videotape, and two animals falling off the maze. See Figure 2 for complementary data.

from control, while animals treated with 10.0 mg/kg maprotiline showed a preference for open arms (40%) over closed arms (27%; $p < 0.01$) with no distinction between centre platform and either type of arm.

Effects of Fluvoxamine

The effects of fluvoxamine are summarized in Table 5. No behavioural changes were evident at 2.0–4.0 mg/kg fluvoxamine while, at the highest dose tested (8.0 mg/kg), the only change observed was a significant increase in percent protected flat back approach behaviour. Follow-up tests on the significant two-factor ANOVA (details above) confirmed the general inactivity of subchronic fluvoxamine in the present model; the spatiotemporal preference (i.e., percent time spent in different maze sections) of fluvoxamine-treated subjects did not differ from controls.

DISCUSSION

The present study examined the effects on plus-maze behaviour in mice of subchronic treatment with a traditional anxiolytic (chlordiazepoxide), a NA-selective reuptake inhibitor (maprotiline), and a 5-HT-selective reuptake inhibitor (fluvoxamine). As the current treatment regimen necessitated daily handling and injection over a period of 21 days, it is important to note that this procedure had minimal effects upon behavioural baselines (Table 2; injected control vs. saline control); interestingly, these data are fully consistent with the minimal effects of handling and injection seen in our parallel study on social behaviour (20). While direct comparisons are not feasible, it is also important to note that the basal plus-maze profile of male CD1 mice differs from that previously observed with DBA/2, T1, and BALB/c mice (10,69). In particular, CD1 mice showed higher levels of open arm entries (resulting in high baseline scores for % open entries) and did not display closed arm returns to a recordable level. Thus, to maintain the sensitivity of the test, our scoring method was adapted to incorporate measures of end-open arm activity which, in other laboratories (15,18), have been found to be sensitive to anti-anxiety agents.

Under the present test conditions, daily treatment with chlordiazepoxide for 21 days produced several behavioural alterations indicative of anxiety reduction. The most prominent changes were evident at the highest dose tested (8.0 mg/kg) and consisted of increases in open arm entries, percent open arm time, and end-open head-dipping together with reductions in total SAP and in the percent protected forms of head-dipping and stretched attend postures. The fact that percent open entries remained unaffected by chlordiazepoxide treatment can be directly attributed to the high baseline for his measure in CD1 mice. These findings are consistent with much previous research, in this laboratory and elsewhere, indicating that the plus-maze is reliably sensitive to both acute and chronic benzodiazepine treatment (63). Furthermore, the observation that SAP-related measures were also affected across the dose range (1.0–8.0 mg/kg) confirms the view that risk assessment behaviour is particularly sensitive to this class of anti-anxiety agent (3,11,67).

In a previous article (12), we reported that plus-maze behaviour in mice is unaffected by acute or chronic treatment with the antidepressant/antipanic agent, imipramine, and suggested that this negative finding may have been related to the compound's relative nonspecific inhibition of monoamine reuptake. In this context, maprotiline is a highly selective

TABLE 5

EFFECTS OF SUBCHRONIC TREATMENT WITH FLUVOXAMINE (2.0-8.0 MG/KG, DAILY 21 DAYS) ON THE BEHAVIOUR OF CD1 MICE IN THE ELEVATED PLUS-MAZE TEST OF ANXIETY

Variable	Saline (n = 14)	2.0 mg/kg (n = 9)	4.0 mg/kg (n = 9)	8.0 mg/kg (n = 10)
Total entries	25.9 ± 2.2	27.6 ± 2.7	24.9 ± 2.9	30.6 ± 2.5
Open entries	14.6 ± 1.8	14.4 ± 2.4	13.4 ± 2.4	14.9 ± 2.2
Closed entries	11.3 ± 1.0	13.2 ± 0.8	11.4 ± 1.4	15.7 ± 1.2
Total rears	20.5 ± 2.4	18.9 ± 1.6	17.4 ± 3.4	19.5 ± 2.6
% Open entries	54.8 ± 3.4	50.0 ± 4.1	52.0 ± 5.3	46.4 ± 5.0
% End-open entries	21.5 ± 1.8	20.2 ± 2.4	19.2 ± 2.7	18.2 ± 2.5
% Open time	26.8 ± 3.4	22.9 ± 2.8	26.2 ± 5.5	21.5 ± 3.0
% End-open time	10.5 ± 1.5	8.9 ± 1.9	10.1 ± 2.2	9.3 ± 1.7
% Centre time	38.5 ± 2.6	34.6 ± 3.0	38.2 ± 4.2	42.7 ± 3.0
% Closed arm time	34.7 ± 2.4	42.5 ± 2.3	35.6 ± 5.3	35.8 ± 1.8
Total head-dips	17.0 ± 1.3	14.0 ± 1.0	16.3 ± 2.9	14.7 ± 1.4
End-open head-dips	4.0 ± 0.7	3.6 ± 0.7	4.2 ± 1.2	3.6 ± 0.5
Total SAP	20.3 ± 1.2	14.1 ± 1.5	16.1 ± 1.7	16.0 ± 1.7
End-open SAP	3.4 ± 0.4	2.5 ± 0.5	3.0 ± 0.7	2.5 ± 0.4
Rearing duration (s)	14.3 ± 1.7	16.6 ± 3.5	13.7 ± 2.6	17.2 ± 3.6
Sniff duration (s)	31.9 ± 3.4	31.9 ± 4.2	27.7 ± 4.5	33.0 ± 4.2
Grooming duration (s)	13.5 ± 3.1	19.8 ± 3.1	19.5 ± 3.4	11.3 ± 1.8
Flat back approach duration (s)	25.3 ± 3.8	32.0 ± 7.3	36.4 ± 5.3	47.1 ± 7.9
%p head-dips	47.9 ± 5.9	48.1 ± 5.7	49.5 ± 8.2	55.6 ± 6.2
%p SAP	60.9 ± 4.0	59.5 ± 6.2	60.9 ± 7.5	67.0 ± 5.2
%p sniff	85.7 ± 2.9	88.2 ± 3.4	90.4 ± 3.1	91.9 ± 2.5
%p flat back approach	22.1 ± 3.8	33.3 ± 4.6	32.9 ± 7.2	42.4 ± 6.8*

* $P < 0.05$; no other comparisons significant.

inhibitor of NA reuptake, while fluvoxamine is one of the more selective inhibitors of 5-HT reuptake currently available (35). Somewhat contrary to expectation, our data show that, of these two antidepressants, only maprotiline demonstrated any anxiolytic-like activity. This was evident at the lowest dose tested (0.5 mg/kg) and comprised significant increases in percent open and end-open arm time, percent end-open entries, and end-open head-dips. This profile overlaps with that seen with chlordiazepoxide with the exception that maprotiline did not appear to affect risk assessment measures such as SAP. These anxiolytic-like effects of were lost at higher doses (2.0–10.0 mg/kg) of maprotiline, with possible motor suppression becoming evident at 10.0 mg/kg, i.e., reduction in rearing. Although present data do not allow for an explanation of the loss of anxiolytic-like activity at higher doses of this NA-selective reuptake inhibitor, they do suggest the potential value of further studies using < 0.5 mg/kg in a range of anxiety models. Against this profile, subchronic treatment with the 5-HT-selective reuptake inhibitor, fluvoxamine, was virtually without behavioural effect under present test conditions. As such, our data point to a commonality in the behavioural effects of chlordiazepoxide and maprotiline, and a major difference in profiles between these compounds and that of the SSRI, fluvoxamine.

It has been assumed for some time that reuptake blockers that have relatively greater efficacy on NA mechanisms would be particularly useful in treating retarded depression, whereas those with greater efficacy on 5-HT mechanisms would be more beneficial in agitated/anxious depression (13). Although by no means a universal finding (53), recent clinical data would not be inconsistent with this view (25,48,73). However, it is clear that SSRIs are most effective in panic disorder with no evidence that NA-selective reuptake inhibitors are effective in this regard (1,21,50,56,72). Furthermore, while SSRIs are not effective in generalized anxiety disorder (40), there is some evidence that patients with anxious-depression do respond favourably to maprotiline (53). This analysis suggests that present results may be a function of the type of anxiety evoked in mice by exposure to the elevated plus-maze. Certainly, our negative finding with fluvoxamine (an established panicolytic agent) is consistent with our previous failures to detect activity in the murine plus-maze with other agents thought to impact panic (e.g., imipramine, CCK-related peptides, CCK receptor antagonists, sodium lactate and isoproterenol, (12,37,64). Recent research on a putative animal model of panic, conditioned ultrasonic vocalisations, would be consistent with this line of reasoning in that fluvoxamine, but not maprotiline, inhibited such distress signals (47). The fact that a number of SSRIs (including fluvoxamine) apparently do produce positive effects in the rat plus-maze following chronic treatment (31,32,44) may suggest that, in rats, exposure to an elevated maze induces a chimera of anxiety-related responses (includ-

ing panic) whereas mice react to such aversive experience with a state more akin to generalized anxiety disorder. Nevertheless, problems of consistency are evident with the rat plus-maze in that: (1) acute treatment with SSRIs can produce enhanced anxiety (2,6,31,32), no effect (44), or even a reduction in anxiety (36); and (2) where anxiolytic-like effects are seen with acute SSRI treatment (36), there is little correlation with drug action on 5-HT reuptake.

In summary, the present direct comparison of the effects of subchronic treatment with chlordiazepoxide, maprotiline and fluvoxamine reveals clear similarities in the behavioural effects of the benzodiazepine and a NA-selective reuptake inhibitor, as well as a major distinction between these effects and those seen with a 5-HT-selective reuptake inhibitor. These findings are remarkably similar to those obtained in our parallel study on the effects of these same compounds on social behaviour in CD1 mice (20). It is suggested that present findings are consistent with the view (12,64) that the murine plus-maze is sensitive only to agents that are effective in the management of generalized anxiety disorder and not those that alter panic-like responses. Finally, although further research on the anxiolytic efficacy of maprotiline is clearly required, our data may suggest a possible noradrenergic basis for the recently demonstrated clinical efficacy of imipramine in patients generalized anxiety disorder (60).

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