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Exploration of Mice in a Black and White Test Box: Validation as a Model of Anxiety

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COSTALL, B., B. J. JONES, M. E. KELLY, R. J. NAYLOR AND D. M. TOMKINS. *Exploration of mice in a black and white test box: Validation as a model of anxiety.* PHARMACOL BIOCHEM BEHAV **32**(3) 777–785, 1989.—The validity of a black and white test box to measure changes in mouse exploratory behaviour relevant to assessment of anxiety was investigated by variation of the illumination within the test box, the use of different strains of mice, holding conditions and drug treatments. The suppression of exploratory activity in the white section caused by bright illumination was antagonised by anxiolytic agents from the benzodiazepine series, buspirone, 5-HT_3 receptor antagonists, alcohol, nicotine, morphine and SCH23390. The anxiogenic agent FG7142 exacerbated the behavioural suppression. Black C57/BL/6, brown DBA_2 and albino BKW mice were sensitive to the effects of drug treatments, whereas albino Tuck mice were less responsive. It is concluded that the characteristic change in mouse exploratory behaviour caused by anxiolytic agents is to preferentially increase exploratory behaviour in the white aversive section of the black and white test box. It is most consistently shown by (a) an increased time spent in the white section with proportional increases in (b) rearings and (c) ambulation and (d) a delay in the initial transition from the white to the black section.

White and black test box Mouse Anxiety model

BEHAVIOURAL paradigms using conflict procedures, social interaction and exploration of novel environments have been widely used as animal models of anxiety (14,17). Based on findings that open fields have aversive properties which inhibit rodent exploratory behaviour (3,21), Crawley and Goodwin (10) described a model in mice where benzodiazepines produced a facilitation of exploratory behaviour between a lighted open field and a dark enclosure. The essential feature was the measurement of increased transitions between the light and dark chambers, the time spent in each compartment remaining the same. The method is simple, rapid and demonstrates a pharmacological specificity for the benzodiazepines (1, 8, 22).

Using a similar light and dark test box system, we have confirmed that diazepam can enhance exploratory activity, particularly rearing and ambulation in the normally aversive light environment. Furthermore, $5HT_3$-receptor antagonists and agents from the substituted benzamide series had similar profiles (4, 6, 7). However, unlike the findings of Crawley and colleagues (8–10, 24), the increased exploratory behaviour was associated with an increased time spent in the light area, transitions between the two compartments remaining unchanged (5). In the present study we investigate in more detail the methodology of the light and dark test box system as a model relevant to the assessment of drug-induced changes in anxiety.

METHOD

Male albino BKW mice (Bradford strain) were used throughout the studies unless stated otherwise. Black C57/BL/6, brown DBA_2 and albino Tuck mice were obtained from Charles River. The mice, weighing 20–30 g, were housed in groups of 10 in conditions of constant temperature (21°C) and controlled lighting (dark period 07.00–19.00 hr, unless otherwise stated) and fed ad lib on a standard laboratory chow.

Tests for changes in behaviour were conducted between 13.00 and 18.00 hr in a quiet darkened room illuminated with a red light. In the majority of experiments mice were taken from a dark holding room in a dark container to the dark testing room where, after a 1-hour period of adaptation to the new environment, they were placed into the test box. The metal test box ($45 \times 27 \times 27$ cm high) was positioned on a bench 1 m above floor level. The box was open-topped and the base lined into 9 cm squares, two-fifths painted black and illuminated [excepting where specified by red light (1×60 W)] and partitioned from the remainder of the box which was painted white and brightly illuminated with a 1×60 W light source, the red and white lights being located 17 cm above the box. The compartments were connected by an opening 7.5×7.5 cm located at floor level in the centre of the partition. In most experiments mice were placed into the centre of the white, brightly lit area and the operator withdrew from the room. The mice were observed by remote videorecording and five behaviours were noted, a) the number of exploratory rearings in the white and black sections, b) the number of line crossings in the white and black sections, c) the number of transitions between the two compartments, d) the time spent in the white and black areas and e) the

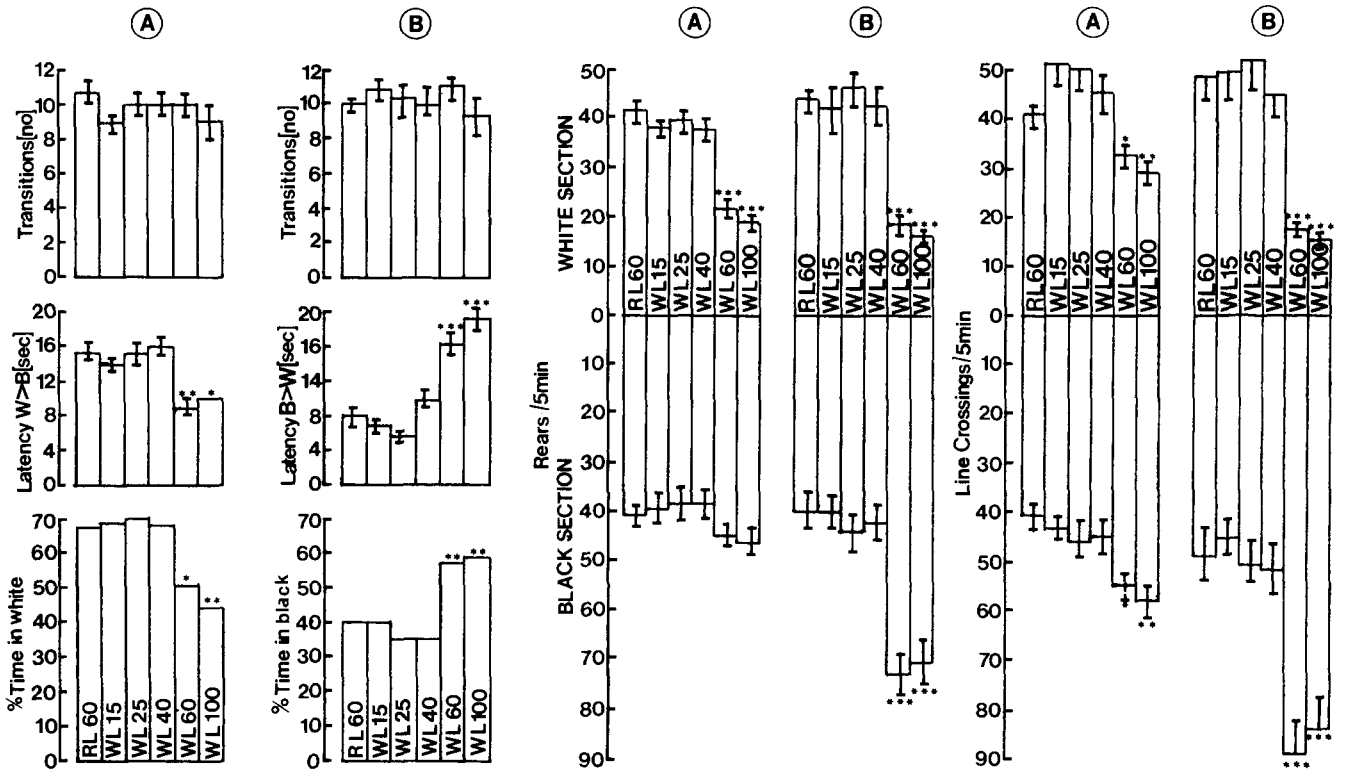

FIG. 1. Effect of changed illumination in the white area of the black and white test box on mouse exploratory behaviour. The illumination in the black section was held constant using red light (60 W) and the illumination in the white section was varied from red lighting (RL, 60 W) to bright white lighting (WL, 15 to 100 W). Albino BKW mice were initially placed into either (A) the white or (B) the black section and measurements of the time spent in and transitions between the two compartments, rearing behaviour and line crossings and latency of the initial movement from the white to the black or black to the white area were made from remote video recordings taken over a 5-min period. Values represent the mean ± S.E.M.s of 5 determinations; S.E.M.s on the original data for calculation of the % time spent in the two compartments were in the range 9.8–12.7%. Significant increases or decreases in responding compared to the values obtained using red light illumination are indicated as $*p<0.05$, $**p<0.01$, $***p<0.001$ (one-way ANOVA followed by Dunnett's *t*-test).

latency of the initial movement from the white to the black area. In some experiments the experimental procedure was varied with respect to the use of reverse lighting conditions in the holding rooms, the nature of the illumination and positioning of the mouse in the test box.

Mice were used once only in treatment groups of 5. The results were analysed using single factor Analysis of Variance followed by Dunnett's procedure for comparing all treatments with control.

(+)Amphetamine sulphate (Sigma), amitriptyline hydrochloride (Merck, Sharp and Dohme), bupropion hydrochloride (BurroughsWellcome), BRL43694 hydrochloride(endo)-N-[9 methyl-9-azabicylo(3,3,1)non-3-yl]-1-methyl-indazole-3 caboxamide (Glaxo), buspirone hydrochloride, caffeine citrate, cocaine hydrochloride (B.D.H.), fluphenazine hydrochloride (Squibb), ethanol (Burroughs Ltd), GR38032F (1,2,3,9-tetra-hydro-q-methyl-3-[(2-methyl-1H-imidazol-l-yl) methyl]d-4H-car-bazol-4-one,hydrochloride·2 H_2O (Glaxo), FG7142 (N-methyl-β-carboline-3-carboxamide, Research Biochemicals Inc.), ICS-205-930 ([3α-tropanyl] - 1H - indole - 3 - carboxylic acid ester, Sandoz), imipramine hydrochloride (Ciba-Geigy), MDL72222([1αH,3α, 5αH-tropan - 3 - yl - 3,5-dichlorobenzoate methyl sulphate hemihydrate (Merrell)], metoclopramide monohydrochloride (Beecham), morphine hydrochloride (B.D.H.), nicotine hydrogen tartrate (B.D.H.), tiapride hydrochloride (SESIF) and triazolam hydrochloride (Upjohn) were dissolved in distilled water, chlordiazepoxide and diazepam (Roche) were dissolved in the minimum amount of polyethylene glycol prepared to volume with distilled water and sulpiride prepared in the minimum amount of hydrochloric acid prepared to volume with distilled water. Alcohol was administered in the drinking water as a 1, 4 and 8% solution and animals tested on the 3rd day. Morphine was infused continuously (0.5, 2.5 and 10 mg/kg/day) from Alzet osmotic minipumps implanted subcutaneously and animals tested on the 4th day. All other drugs were administered 40–45 min before testing and were injected intraperitoneally in a volume of 1 ml/100 g.

RESULTS

The Effect of Changes in Illumination of the Test Box on Mouse Exploratory Behaviour

The black painted compartment was illuminated with a constant red light (60 W) and the light intensity varied in the white painted area. If red light (60 W) was also used to illuminate the white area, albino BKW mice spent 60 to 70% of their time in the white section coincident with its greater size. The rearing behaviour of mice usually occurred against the walls of the box and was of equal intensity in both sections (40–50 rears/5 min), similar comments applying to line crossings (40–50 crossings/5 min). This behavioural profile occurred when mice were placed initially

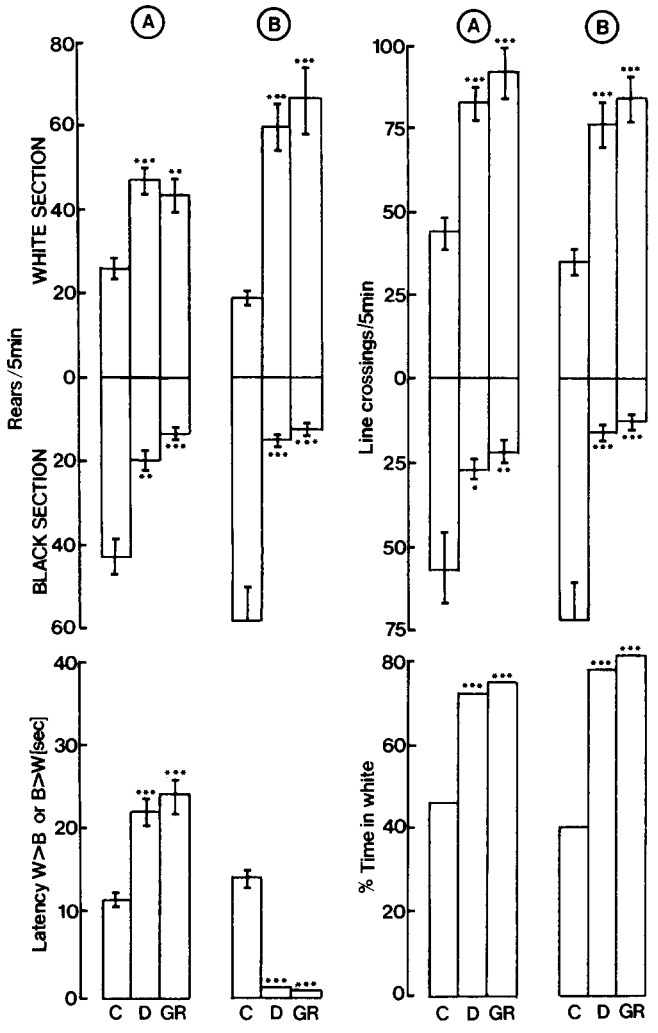

FIG. 2. Effects of diazepam and GR38032F on the behaviour of the albino BKW mouse in the white and black test box system. Mice received diazepam (D, 1.25 mg/kg) or GR38032F (GR, 0.001 mg/kg) and were placed into the centre of (A) the white section (illuminated with white light, 60 W) or (B) the black section (illuminated with red light, 60 W). Measurements of rearing behaviour, line crossings and the % of time spent in each section and the latency of the first movement from one compartment to the other were made from remote video recording taken over a 5-min period. Values represent the mean ± S.E.M.s of 5 determinations; S.E.M.s on the original data for calculation of the % time spent in the compartments were in the range 7.3–10.9%. Significant increases or decreases in responding compared to control (C, vehicle-treated) mice are indicated $*p<0.01$, $**p<0.001$ (one-way ANOVA followed by Dunnett's *t*-test).

into the centre of the black or white arena (Figs. 1 and 2).

The replacement of red illumination in the white area by a white light source of 15 or 40 W caused no significant change in the profile of exploratory activity. However, the use of a 60- or 100-W light source modified exploratory activity in a comparable manner, mice showing fewer rearings and line crossings in the white section (decreases of 53 to 63%) with increased values in the dark section. For mice initially placed into the black area, the increases in rearings and line crossings in this area (53%) were greater than could be accounted for by the 32% increase in time spent in the area: such mice showed an absolute increase in

TABLE 1

ILLUMINATION AND TEMPERATURE IN THE BLACK AND WHITE SECTIONS OF THE TEST BOX FOLLOWING CHANGES IN LUMINOSITY IN THE WHITE AREA

Illumination in the White Area	Light Intensity (lux)		Temperature	
	Dark Area	White Area	Dark Area	White Area
Red 60 W	0	10	22.4	
White 15 W	0	40	22.3	
White 25 W	0	160	22.0	
White 40 W	0	240	21.9	
White 60 W	0	400	22.6	
White 100 W	20	600	24.9	25.8

A red or white light source was located 17 cm above the white section, a constant 60-W red light source was located 17 cm above the black section.

rearings and line crossings. The decrease in rearings and line crossings of such mice in the white area was also greater than could have been predicted from the modest 31–35% reduction in time spent in this area. Under the conditions of bright illumination in the white area, mice placed into the white area showed a reduced latency in moving into the black area, i.e., from an order of 15 sec to 9–10 sec. Conversely, mice initially placed into the black area would move within 8 sec into the white area under red/red light conditions, this latency was increased 200 to 238% in the presence of 60–100-W white illumination in the white area (Fig. 1). There were no changes in the total transitions between the white and black sections in any of the above paradigms (Fig. 1). The totality of the changes in behaviour suggests that conditions of high illumination in the white area have aversive consequences on mouse exploratory behaviour.

The measurements of the illumination in the black and white sections, and temperature changes, are indicated in Table 1. There was a small increase in temperature using the 100-W light source. Since a 60-W light source was as effective as a 100-W source in causing behavioural change, and failed to modify temperature, the use of 60-W light source was adopted in all subsequent studies.

The Effect of Diazepam and GR38032F on Mouse Behaviour in the White:Black Test Box

Doses of diazepam (1.25 mg/kg) and GR38032F (0.001 mg/kg) were selected on the basis of preliminary studies as those which caused maximal changes in behaviour. Male albino BKW mice were placed into the centre of the white or black section 40–45 min after drug treatment. The control (vehicle-treated) mice placed into the black or white section showed greater rearing and line crossing behaviour in the black section of the test box, caused by the high illumination (60 W) in the white section. Treatment with diazepam and GR38032F caused an increase in rearings and line crossings in the white section and a reduction in the black section (Fig. 2). Also, animals placed into either the white or black areas of the test box spent more time in the brightly-lit white area. In addition, mice placed into the white area showed a significant delay in moving from the white to the black area. Conversely, mice placed into the black area moved almost at once into the white area. The drug treatments failed to modify the number of transitions between the two compartments (Fig. 2).

A Comparison of the Responses of Black C57/BL/6, Brown DBA_2, Albino BKW and Albino Tuck Mice to Diazepam and GR38032F in the Black and White Test Box

The overall profile of response of the black C57/BL/6, brown

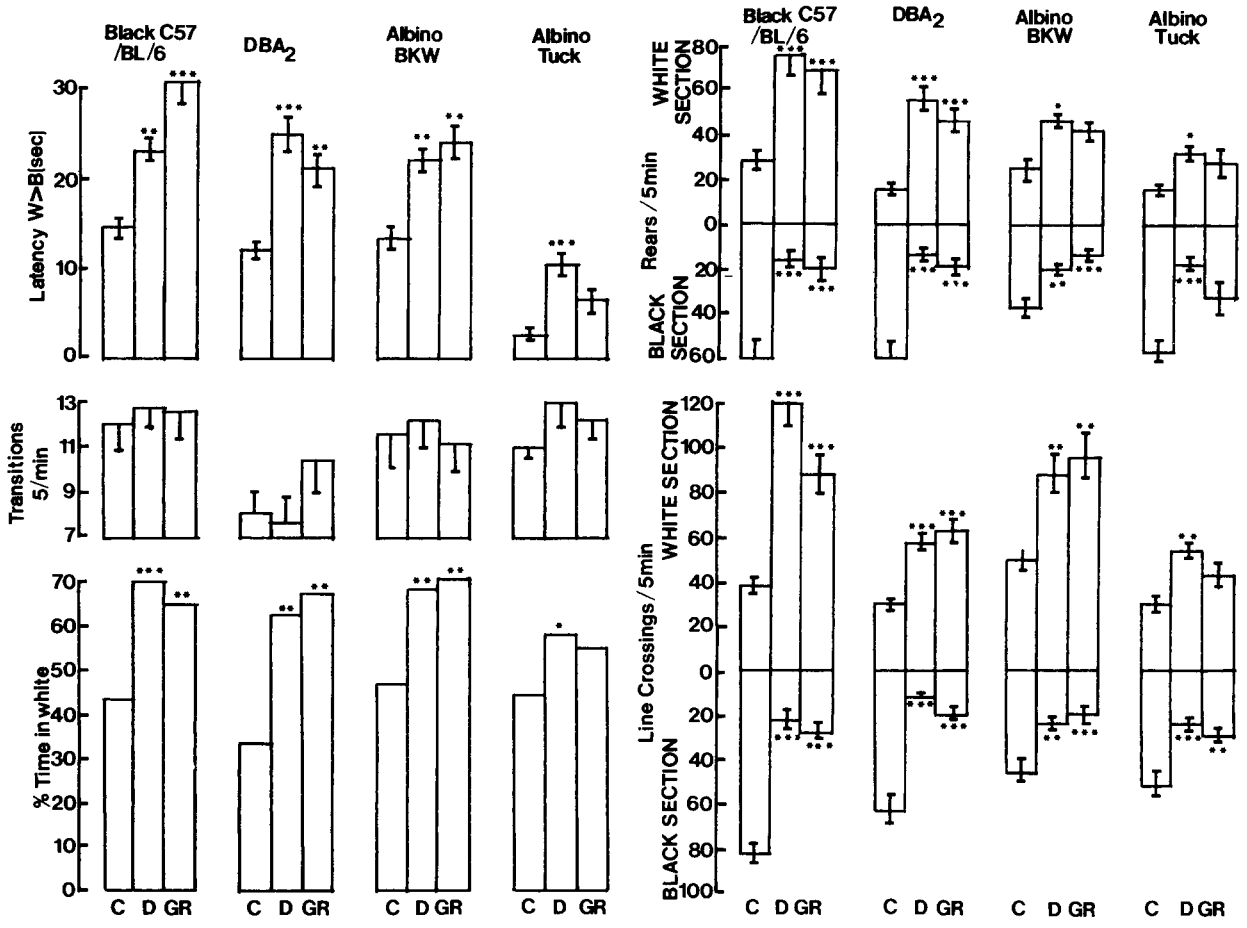

FIG. 3. The effects of diazepam and GR38032F on the behaviour of black C57/BL/6, brown DBA_2, albino BKW and albino Tuck mice in the black and white test box. Mice received diazepam (D, 1.25 mg/kg) or GR38032F (GR, 0.001 mg/kg) and were placed into the centre of the white area (illuminated with white light, 60 W), the dark section being illuminated with red light (60 W). Measurements of rearing behaviour and line crossings, the % time spent in each section, the transitions between the two compartments and the latency of the first movement from the white section were made from remote video recordings taken over a 5-min period. Values represent the mean ± S.E.M.s of 5 determinations; S.E.M.s on the original data for calculation of the % time spent in the compartments were in the range 8.0–11.2%. Significant increases or decreases in responding compared to control (C, vehicle-treated) animals are indicated as $*p<0.05$, $**p<0.01$, $***p<0.001$ (one-way ANOVA followed by Dunnett's *t*-test).

DBA_2, and albino Tuck mice to diazepam and GR38032F was as described above for albino BKW mice. However, between the 4 strains there were quantitative differences in responses. Thus, the black C57/BL/6 mice showed the greatest increases in rearings and line crossings in the white section, the brown DBA_2 and albino BKW mice also showed significant changes, but the increases recorded in the albino Tuck mice were less marked (Fig. 3). Such changes were mirrored by the latency of the first movements from the white to the black section: control-treated black C57/BL/6 mice moved into the black section after 14.5 ± 1.6 sec and this was increased to 23 ± 2.0 to 31 ± 2.9 sec after treatment with diazepam and GR38032F. The brown DBA_2 and albino BKW mice showed similar changes and all three strains showed an increased time spent in the white area. In contrast, the control albino Tuck mice moved rapidly (2.5 ± 0.8 sec) into the black area, with modest increases in the time spent in the white area after treatment with diazepam (Fig. 3). From the data presented on Fig. 3 it is clear that in no strain of mouse were transitions altered by the drug treatments.

Responses of Mice Housed in Light at the Time of Testing

Albino BKW mice were housed in light conditions between 08.00 and 20.00 hr for 2 weeks and then taken from the holding room to the dark test room. Such mice failed to exhibit aversion to the white environment. Thus, mice placed into the black section of the test box demonstrated a similar number of rears and line crossings in white and black sections (32 ± 3.6 and 38.0 ± 4.2 rears/5 min; 39 ± 4.4 and 44.6 ± 3.5 line crossings/5 min): similar values were recorded if mice were placed into the white section.

Mice placed into either the white or black section of the test box after treatment with diazepam (1.25 mg/kg) or GR38032F (0.001 mg/kg) performed similarly to controls, e.g., for mice placed into the white section values for rearing behaviour in the white section, for vehicle-diazepam- and GR38032F-treated animals were respectively 38.5 ± 4.1, 42.0 ± 3.9 and 37.0 ± 3.2 rears/5 min. The drug treatments also failed to modify the % time spent in the black area after mice were placed in to the white section. For vehicle-, diazepam- or GR38032F-treated animals the values were respectively 52, 51 and 53%; similar results were found for animals placed into the black section. The latency of mice moving from the white to the black was also unchanged; e.g., the values for vehicle-, diazepam- and GR38032F-treated animals were respectively 12 ± 2, 14.5 ± 1.8 and 12 ± 1.4 sec and for movement from the black to the white 4.8 ± 0.4, 4.0 ± 0 and

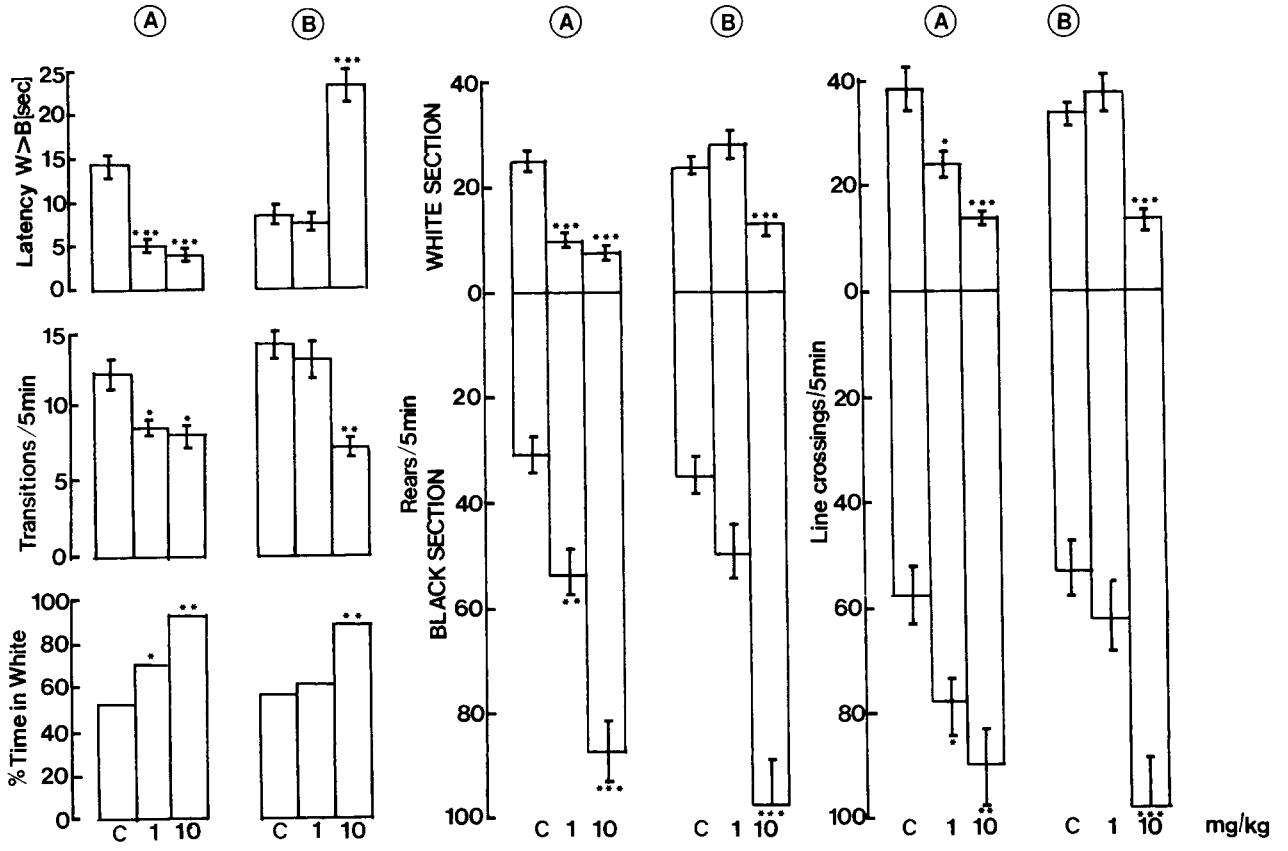

FIG. 4. The effect of FG7142 on the behaviour of albino BKW mice in the black and white test box. Mice received FG7142 or vehicle (Control, C) and were placed into the centre of the (A) white area (illuminated with white light, 60 W) or (B) black area (illuminated with red light, 60 W). Measurements of rearing behaviour and line crossings, the transitions between the two compartments, the % time spent in the white or black section and the latency of the initial movement from one area to the other were made from remote video recordings taken over a 5-min period. Values represent the means ± S.E.M.s of 5 determinations; S.E.M.s on the original data for calculation of the % time spent in the two compartments was in the range 9.3–12.5%. Significant increases or decreases in responding compared to controls are indicated as $*p<0.05$, $**p<0.01$, $***p<0.001$ (one-way ANOVA followed by Dunnett's t-test).

5.7 ± 0.6 sec. The drug treatments also failed to modify transitions which were generally in the range of 10–12/5 min.

The Effect of FG7142 on Mouse Behaviour in the Black and White Test Box

Albino BKW mice treated with FG7142 (1 and 10 mg/kg) and placed into the white area exhibited enhanced exploratory behaviour in the black area with corresponding decreases in the white. Behaviour was changed in a similar manner by 10 mg/kg FG7142 when mice were placed into the black section (Fig. 4). The increased rearings and line crossings were associated with an increase in time spent in the black area, a decrease in the latency of moving from the white to the black section and an increased latency of movement from the black to the white section. In addition, such changes were accompanied by a decrease in the transitions between the two compartments (Fig. 4).

Comparison of the Abilities of Psychopharmacological and Other Agents to Modify Mouse Behaviour in the Black and White Test Box

Using albino BKW mice and 60-W illumination in the white section, the full profile of action of a dose range of diazepam to enhance rearings and line crossings in the white section (with corresponding reductions in the black), to increase the time spent in the white section and increase the latency of the first movement from the white to the black section is shown in Fig. 5. Comprehensive dose ranges of other compounds were assessed and detailed results for a neuroleptic agent fluphenazine, an antidepressant agent amitriptyline and the stimulant amphetamine are presented on Fig. 5. For other compounds, representative data for the effect of drug treatments on rearing behaviour alone is given on Fig. 6.

The profile of action of compounds from the benzodiazepine series (diazepam, triazolam and chlordiazepoxide), the substituted benzamide series (sulpiride, tiapride and metoclopramide), buspirone, the 5-HT_3 receptor antagonists (BRL 43694, ICS-205-930, GR38032F, zacopride and MDL72222) and also alcohol, nicotine and morphine was identical to that of diazepam, increasing exploratory activity and time spent in the white section with a decrease in the black (Fig. 6). Phenobarbitone, SCH23390 and bupropion caused a similar spectrum of behavioural change although the intensity of the change was not as marked (Fig. 6).

This profile of action was not observed using the other agents. Thus, fluphenazine reduced rearings and line crossings in both areas and the number of transitions between the two sections. The motor depressant effects of fluphenazine, reflected in the above data, may also have contributed to the delay in moving from the white to the black section. Haloperidol had a similar effect.

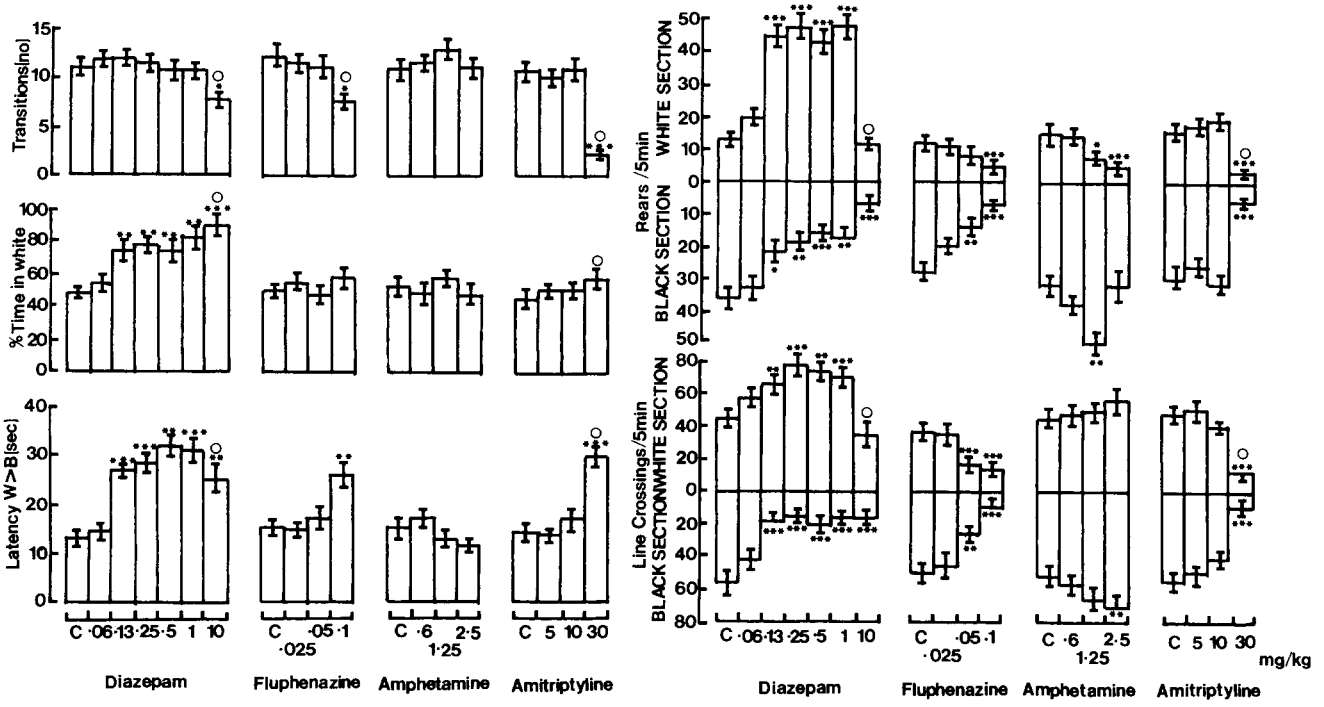

FIG. 5. The effect of diazepam, fluphenazine, amphetamine and amitriptyline on the behaviour of albino BKW mice in the black and white test box. Mice received the injection of drug or vehicle (Control, C) and were placed into the centre of the white area (illuminated with white light, 60 W), the black area was illuminated with red light (60 W). Measurements of rearing behaviour and line crossings, the transitions between the two compartments, the % of time spent in the white or black section and the latency of the initial movement from the white to the black area were made from remote video recordings taken over a 5-min period. Values represent the means ± S.E.M.s of 5 determinations; S.E.M.s on the original data for calculation of the time spent in the two comparments was in the range 7.7–12.3%. Significant increases or decreases in responding compared to controls are indicated as $*p<0.05$, $**p<0.01$, $***p<0.001$ (one-way ANOVA followed by Dunnett's *t*-test). ○ = Sedation.

Amphetamine treatment lead to reduced rearings in the white section with increases in the black section, without changes in line crossings or the time spent in either the white or black area, or the transitions between the compartments or the latency of movement from the white to the black section. Amitriptyline failed to modify rearings, line crossings, % time spent in the black or white sections, transitions or latency of movement from the white to the black sections excepting at the high doses of 30 mg/kg which caused sedation leading to reductions in all components of exploratory behaviour. This was also observed using a high dose of imipramine (20 mg/kg) and diazepam (10 mg/kg) which obscured an interpretation of the changes in exploratory behaviour. Cyproheptadine, ritanserin and methysergide similarly caused nonspecific changes in exploratory behaviour at high doses (Fig. 6). Caffeine failed to consistently modify exploratory rearings and line crossings in either the black or white sections (Fig. 6), although there was a trend to reduce the latency of movement from the white to the black section. Cocaine enhanced exploration in the black section with a decrease in the white (Fig. 6).

DISCUSSION

Measurement of changes in exploration of mice as measured in a light and dark test box would appear to offer a particularly simple model of anxiety (10), the method being based on the observation that whilst rodents tend to explore a novel environment open fields appear to have aversive properties which may inhibit their exploratory behaviour (3, 21, 25). In the present investigation the exploratory behaviour of mice within a black and white two compartment system was found to be dependent on a number of factors.

If mice are removed from the holding room during their dark cycle in a dark container to the dark test room, and placed into the test box where both sections are illuminated with a red light, they demonstrate a random activity within the two compartments. Thus, mice spent approximately 60–70% of their time in the white area and 30% in the black area, which appear to reflect the relative sizes of the two chambers. This pattern of exploratory behaviour continued if the red illumination in the white section was replaced with a relatively low level of white illumination of 10 to 240 lux. However, 400 lux illumination in the white area was sufficiently aversive to significantly reduce the time spent and the rears and line crossings in the white section, with corresponding increases in the black. Further, the time taken for the initial movement of mice from the white section to the black was reduced by 50%;conversely, animals placed initially into the black section delayed their movement into the white section. Taken together, these findings indicate that a brightly lit white environment is aversive to mice and inhibits their exploratory behaviour. It is emphasised that this response is obtained from mice taken and tested during the dark period of their lighting schedule, since mice taken during their light period failed to respond to the aversive stimulus.

The aversion to the brightly lit white area was antagonised by the benzodiazepines diazepam, chlordiazepoxide and triazolam (the benzodiazepine antagonist Ro15-1788 was inactive), the substituted benzamides sulpiride and tiapride, the 5-HT_3 receptor antagonists GR38032F, ICS-205-930, MDL72222, zacopride and BRL43694, and buspirone, alcohol, nicotine and the selective

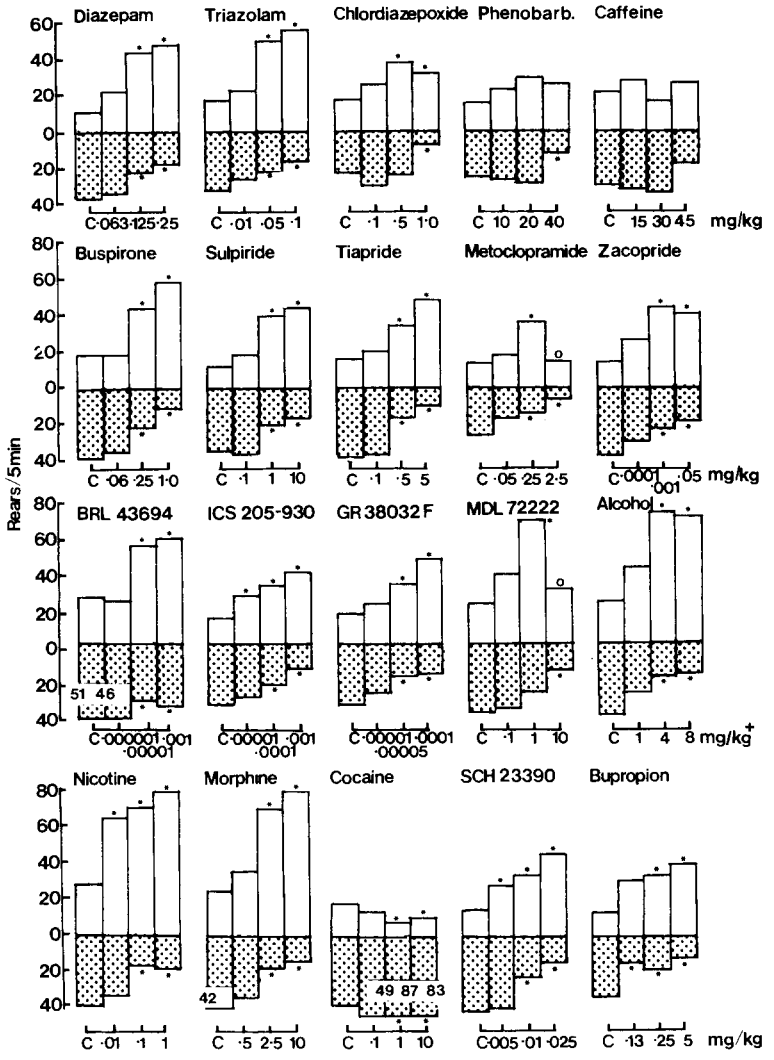

FIG. 6. The effect of benzodiazepines, 5-HT_3 receptor antagonists and other agents on the behaviour of albino BKW mice in the black and white test box. Mice received the injection of drug or vehicle (Control, C) and were placed into the centre of the white area illuminated with white light (60 W), the black area was illuminated with red light (60 W). From behavioural measurements made from remote video recordings taken over a 5-min period, rearing behaviour in the black (stippled histograms) and white sections (open histograms) is presented. Values represent the means ± S.E.M.s of 5 determinations. Significant increases or decreases in responding compared to controls are indicated as $*p<0.05$–0.001 (one-way ANOVA followed by Dunnett's t-test). ○ = Sedation, + = alcohol was administered as a 1, 4, and 8% solution in the drinking water and animals tested on the 3rd day.

dopamine receptor antagonist SCH23390. Thus, the reduced rearings, line crossings and time spent in the white area were reversed by such compounds, the rearings and time spent in the white area returning to values normally shown by mice placed into a test box illuminated by red light in both compartments: indeed, line crossings were actually increased to values above those recorded using the total red light illumination. The disinhibition of the suppression of behaviour was also shown by the time taken for mice to move from or to the white section. Thus, control mice placed into the brightly lit white section would move rapidly into the black area; mice placed in the black area would show a delay in moving into the white (as compared to mice used in conditions of total red illumination). After the drug treatments the apparent apprehension of remaining in or moving to the white area was abolished and, indeed, the delay for the animals to move from the white section, or the speed of moving from the black section, showed that aversion was reduced to levels below that of normal mice placed in conditions of total red illumination.

The ability of diazepam and other agents to enhance exploratory behaviour in the white section was associated with decreased behaviour in the black section. This 'selective' increase in exploratory activity in the white section would argue against the effects of such compounds being mediated via a general increase in locomotor activity. Furthermore, placing mice into a single chamber after treatment with, for example, diazepam or GR38032F does not increase locomotor activity [Costall *et al.* unpublished data, see also (8)]. Whilst increased locomotion is integral to the experimental paradigms investigating changes in exploratory be-

haviour (2, 14, 19, 20), the increased locomotor activity as measured in the black and white test box appears to be a function of an increased exploratory tendency.

The ability of the benzodiazepines and buspirone to modify mouse behaviour in the test box may reflect their anxiolytic action (16,22). Similar comments may apply to the effects of sulpiride and tiapride (15,23). The 5-HT_3 receptor antagonists, alcohol and nicotine, are also active in rat and primate models indicative of anxiolytic activity [(5, 6, 18), Costall *et al.*, unpublished data]. If this hypothesis is correct, then an agent such as FG7142, with known anxiogenic action in the clinic (12), should have the opposite effect to diazepam and the other compounds in the mouse model. This was confirmed since mice treated with FG7142 and placed into the brightly lit white area showed a reduced latency in moving into the black section, an increased time spent in the black section with markedly increased rears and line crossings in this area. All such measures were markedly decreased in the white section. Further, FG7142 reduced the number of transitions between the two compartments, the only occasion in the present studies that changes in this parameter occurred in the absence of sedation.

It is apparent that anxiolytic and anxiogenic agents alter mouse exploratory activity in the white and black test box in an opposite manner. Further, the profiles of action to selectively increase or decrease behaviour in the two compartments were not observed using the neuroleptic agents fluphenazine and haloperidol which decreased motor behaviour in both sections, or the antidepressant agents amitriptyline and imipramine and the 5-HT_1 and 5-HT_2 receptor antagonists methysergide, cyproheptadine and ritanserin, which only modified exploration nonselectively at high sedative doses. Also, amphetamine, a stimulant drug, reduced rearings and line crossings in the white section with increases in the black: the significance of these observations remains to be established. Such findings indicate the pharmacological specificity of the mouse model and are generally consistent with the data reported by Crawley (8). It remains interesting that the selective D-1 dopamine receptor antagonist SCH23390, and the antidepressants nomifensine and bupropion similarly to the anxiolytic agents antagonised, whereas cocaine increased the exploratory behaviour, and is deserving of further study.

In their use of the mouse model, Crawley and colleagues (8,11) have described an increased number of transitions between the two compartments as a key index of anxiolytic action. Further, whilst correlations were described between increases in exploratory rearings, locomotor activity and transitions (8), anxiolytic agents failed to modify the amount of time spent in the white or black areas (10). Whilst a consistent finding in both the present study and that of Crawley and colleagues is the benzodiazepine suppression of the inhibition of rearings and line crossings in the brightly illuminated section, contrasts are clearly found with respect to transitional data and the time spent by mice in the two compartments. A number of factors may contribute to the discrepancies.

The mouse strain may be an important variable. Crawley and colleagues use male NIH albino general purpose mice, whereas Bradford bred male albino BKW mice were used in the majority of our experiments. Crawley and Davis (9), in an investigation of 5 strains of mice, found that basal exploratory activity predicted anxiolytic responsiveness to diazepam. In the present experiments we investigated 4 strains of mice in an attempt to assess strain differences and found quantitative but not qualitative differences in responsiveness. Thus, the most pronounced behavioural changes (rearings, line crossings, % time spent in the two areas and latency of initial movement from one compartment to the other) caused by diazepam and GR38032F were recorded in black C57/BL/6 mice; brown DBA_2 and albino BKW mice also showed significant changes in behaviour, whereas albino Tuck mice showed little response. Whilst differences in results may partly reflect the use of different species, methodological differences may also be of significance. Thus, in contrast to the present test box painted black (⅔) and white (⅗), the polypropylene test box employed by Crawley and colleagues is ⅓ painted black, the remainder non-painted but 'highly' illuminated, the animals remaining in the test box twice as long as in the present experiments.

In summary, using mice in a black and white test box, anxiolytic agents increased exploratory behaviour that was suppressed by the aversive environment; the anxiogenic agent FG7142 caused the opposite effect. The simplicity of the model may provide a rapid means for the assessment of the actions of agents capable of modifying anxiety.

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