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The free-exploratory paradigm: an effective method for measuring neophobic behaviour in mice and testing potential neophobia-reducing drugs

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When given the opportunity to choose between a novel and a familiar compartment (free-exploratory paradigm), BALB/c mice exhibited a preference for familiar places and a marked number of attempts at entry into the novel compartment followed by avoidance responses. In contrast, C57BL/6 mice showed a preference for novel places and very few avoidance responses towards novelty. When novelty was reduced by two familiar odours, fresh sawdust or urine of conspecifics, the neophobia of the BALB/c mice was reversed and the animals clearly showed a preference for the novel compartment. This experimental paradigm can be proposed as an effective animal model for investigating drugs potentially able to reduce neophobia in BALB/c mice. The effects of anxiolytics, effective in the usual animal models of "state" anxiety, were investigated in the free-exploratory paradigm which may model another type of anxiety termed by Lister (1990) "trait" anxiety. Thus, the behavioural effects of two benzodiazepine full agonists, chlordiazepoxide and diazepam, two non-benzodiazepine partial agonists at benzodiazepine receptors, Ro 19-8022 and alpidem, the 5-HT_{1A} receptor agonist, 8-OH-DPAT, and the 5-HT_3 receptor antagonist, zacopride, were assessed in BALB/c and C57BL/6 mice. Chlordiazepoxide, diazepam and Ro 19-8022 completely reversed the preference of BALB/c mice for the familiar compartment, treated animals exhibiting a significant preference for novel places. In contrast, alpidem, 8-OH-DPAT and zacopride did not significantly modify their behaviour. Moreover, the same drugs did not modify the specific responses of C57BL/6 mice toward novelty. These results demonstrate that drugs which bind in a non-selective manner to heterogeneous benzodiazepine recognition sites were very effective in reducing neophobia in BALB/c mice, whereas 5-HT-interacting drugs were unable to counteract their neophobic behaviour. Thus, the free-exploratory paradigm can be proposed as an effective method for testing potential neophobia- ("trait" anxiety) reducing drugs.

Keywords: Alpidem – BALB/c and C57BL/6 mice – Benzodiazepines – Chlordiazepoxide – Diazepam – Free exploration – Neophobia – 8-OH-DPAT – Ro 19-8022 – Serotonin – "Trait" anxiety – Zacopride

INTRODUCTION

It is well documented that novelty can produce a pattern of defensive reactions comparable to those induced by threatening situations (Russell, 1973; Blanchard *et al.*, 1974). Unfamiliar environments elicit several kinds of behavioural and physiological changes in animals: inhibition of ongoing behaviours, increase in arousal, signs of strong emotional reactions such as increase in heart rate, skin conductance, urination, defecation and plasma corticosteroid levels (Archer, 1973; Walsh and Cummins, 1976). However, these responses have been observed in studies in which animals were "forced" into a novel environment and certain authors have questioned whether the so-called fear reactions might be elicited by such "forced" situations rather than by novelty *per se* (Welker, 1957). For example, it is well known that rats and mice, when given the opportunity to move around freely in simultaneously presented novel and familiar compartments in a choice task such as the prototypical paradigm described by Hughes (1968), often exhibit approach responses towards novelty, described as curiosity or novelty-seeking behaviour, followed by a marked preference for the novel places and by strong exploratory responses (Hughes, 1968; Misslin and Ropartz, 1981). In this experimental situation, Swiss mice did not present the usual physiological and endocrine signs of fear (Misslin *et al.*, 1982). However, using the same test, these signs reappeared when animals were unable to regulate their own approach towards novelty, either because they were prevented from returning to the familiar compartment once they had freely entered the novel one, or because they were placed directly by the experimenter into the novel compartment (Misslin and Cigrang, 1986). These latter results clearly demonstrate

that fear is induced by compelling and stressful components of the experimental situation rather than by novelty *per se*. It has therefore been suggested that in several animal models of anxiety, described as tests of exploratory behaviour, such as the staircase test (Thiébot *et al.*, 1973), the elevated plus-maze (Pellow *et al.*, 1985) or the light/dark choice procedure (Crawley and Goodwin, 1980; Costall *et al.*, 1989; Misslin *et al.*, 1989), in which animals are forced into a novel environment, they actually attempt to escape from these places rather than exhibit exploration (Misslin, 1983). It is well known that in such situations, which usually involve "state" anxiety (Lister, 1990), anxiolytic drugs have often been considered to increase the so-called exploratory responses (for a recent review, see Sanger, 1991), whereas in reality they reduced the behavioural inhibition induced by the aversive properties of the constraining elements inherent in these animal models.

The present experiments were designed first to examine the behaviour of BALB/c and C57BL/6 mice, known respectively as "emotional" and "non-emotional" strains (Oliverio *et al.*, 1973; Peeler and Nowakowski, 1987), in a free-exploratory paradigm devoid of compelling components. Since neophobia was found in BALB/c, but not in C57BL/6 mice, we further investigated novelty as the determining factor in the releasing of neophobia in BALB/c mice. Two familiar odours in the novel compartment, fresh sawdust and urine of unfamiliar conspecifics, were used to verify the specific role of novelty in the responses of both strains of mice (Experiment 1).

A second issue was to investigate the effects of some anti-anxiety drugs, known to be effective in animal models of "state" anxiety, on the behaviour of BALB/c and C57BL/6 mice in the free-exploratory test. In contrast to "state" anxiety, the neophobia of BALB/c mice revealed by this situation seems to correspond to the definition given by Lister (1990) of "trait" anxiety rather than to that of "state" anxiety. The following drugs were used: the classical full agonists at benzodiazepine receptors, chlordiazepoxide and diazepam, two non-benzodiazepine partial agonists at benzodiazepine receptors, a quinolizinone, Ro 19-8022 (Jenck *et al.*, 1992), and an imidazopyridine, alpidem (Zivkovic *et al.*, 1990), the 5-HT_{1A} receptor agonist, 8-OH-DPAT, and the 5-HT_3 receptor antagonist, zacopride.

METHODS

Subjects

Male BALB/cByJIco and C57BL/6JIco mice from the Centre d'élevage IFFA CREDO (France), 10 weeks of age at time of testing, were used. Prior to experimental testing, they were housed five to a standard cage containing a constant supply of food pellets and water, and kept on a 12 h reversed light-dark cycle with light on at 20.00 h so that we could observe animals in their active period which is between 14.00 h and 17.00 h. Each mouse was tested only once in one experiment.

Apparatus and procedure

The apparatus consisted of a polyvinylchloride box (30 × 20 × 20 cm) covered with Plexiglas and subdivided into six equal square exploratory units, which were all interconnected by small doors. It could be divided in half lengthwise by closing three temporary partitions. The apparatus was kept on a stand in the mouse room. The experimenter always stood next to the box in the same place. Approximately 24 h before testing, each subject was randomly placed in one half of the apparatus with the temporary partitions in place, in order to be familiarized with it. The floor of this half only was covered with fresh sawdust and the animal was given unlimited access to food and water during the familiarization phase. The duration of this period was 24 h. On the test day, the temporary partitions between the familiar and novel compartments were removed, and the subject was then observed, under red light, for 10 min. Measures were taken of the time spent in the novel half (novelty preference), the number of units entered (locomotion), the number of rears made by the animals (rears) and the number of approach responses towards the novel compartment (mouse poking its nose into novel places, sniffing, sometimes putting its forepaws through doors) followed by avoidance reactions towards novelty (attempts).

Experiment 1

BALB/c and C57BL/6 mice were randomly allocated to three groups, A, B and C ($n = 10$). Mice of Group A were observed under standard conditions, that is with an empty novel compartment; Group B mice were confronted with the novel compartment with fresh sawdust; Group C mice were tested after the novel compartment had been rubbed with the urine of unfamiliar conspecifics.

Experiment 2

Mice of each strain were randomly allocated to the following groups: (a) chlordiazepoxide (0, 2.5, 5, 10 and 20 mg/kg); (b) diazepam (0, 0.25, 1 and 4 mg/kg); (c) Ro 19-8022 (0, 0.25, 0.50, 1 and 2 mg/kg); (d) alpidem (0, 2.5, 5, 7.5 and 10 mg/kg); (e) 8-OH-DPAT (0, 0.016, 0.062, 0.25 and 1 mg/kg); (f) zacopride (0, 0.0001, 0.001, 0.01, 0.1 and 1 mg/kg).

For BALB/c mice tested with chlordiazepoxide $n = 35$ (vehicle) or 15 (drug). For all other groups $n = 10$.

All animals were individually observed under standard conditions, that is with an empty novel compartment.

Drugs

Chlordiazepoxide, 8-OH-DPAT (gifts from Hoffmann-

La Roche, Basle, Switzerland) and zacopride (a gift from Servier, Paris, France) were dissolved in saline. Diazepam and Ro 19-8022 (Hoffmann-La Roche, Basle, Switzerland) as well as alpidem (Synthélabo, Bagneux, France) were suspended in saline with a drop of Tween 80. Drugs were administered i.p. 30 min before testing, in concentrations giving an injection volume of 10 ml/kg, except 8-OH-DPAT which was administered 20 min before testing.

Statistical analysis

Comparisons between groups were made using either a combined analysis of variance followed by a Bonferroni's *a posteriori* *t*-test, if groups came from populations with homogeneous standard deviations, or a combined Kruskal-Wallis non-parametric ANOVA test followed by a Dunn's multiple comparisons *t*-test, if groups came from populations with heterogeneous standard deviations.

RESULTS

Experiment 1

ANOVA revealed significant differences among groups only in BALB/c mice, for time ($KW = 19.5$, $p < 0.001$), locomotion ($KW = 21.5$, $p < 0.001$), rears ($KW = 19.5$, $p < 0.001$) and attempts ($KW = 18.9$, $p < 0.001$). There was also a significant negative correlation coefficient (Bravais-Pearson) in BALB/c control mice (Condition A) between the number of attempts at entry to the novel compartment and time ($r = -0.79$), locomotion ($r = -0.59$) and rears ($r = -0.75$), which suggests that BALB/c mice exhibited so many unsuccessful entry attempts into novel places that their locomotion and rears were lowered. Figure 1 shows that in Conditions B and C, time spent in the novel compartment, locomotion and rears was significantly increased for BALB/c mice, while the number of attempts was decreased (Dunn's multiple comparisons *t*-test). In addition, the sign test revealed that in Condition A, BALB/c mice exhibited a global preference for the familiar compartment ($p < 0.01$) whereas in Conditions B and C they showed a significant preference for the novel compartment ($p < 0.001$). C57BL/6 mice exhibited a global preference for the novel compartment in Condition A ($p < 0.05$) and in Conditions B and C ($p < 0.01$). ANOVA did not reveal any differences among groups in C57BL/6 mice for time [$F(2,27) = 2.7$], locomotion [$F(2,27) = 0.1$], rears [$F(2,27) = 0.1$] and attempts [$F(2,27) = 1.8$].

Experiment 2: BALB/c

Effects of chlordiazepoxide. Analysis of variance revealed significant differences among groups for time [$KW = 42.2$, $p < 0.001$], locomotion [$KW = 44.6$, $p < 0.001$], rears [$KW = 47.4$, $p < 0.001$] and attempts [$KW = 62.4$, $p < 0.001$]. Figure 2 shows that chlordiazepoxide significantly increased time, locomotion and rears, while it decreased the number of attempts. The sign test indicated that control mice exhibited a significant preference for the familiar compartment ($p < 0.001$), while mice treated with 5 and 10 mg/kg exhibited a significant preference for the novel compartment ($p < 0.001$ and 0.05, respectively). Finally, the dose of 20 mg/kg induced a general disorganization in the behaviour insofar as all the parameters tended to be reduced, which probably can be related to the sedative properties of this drug at high doses.

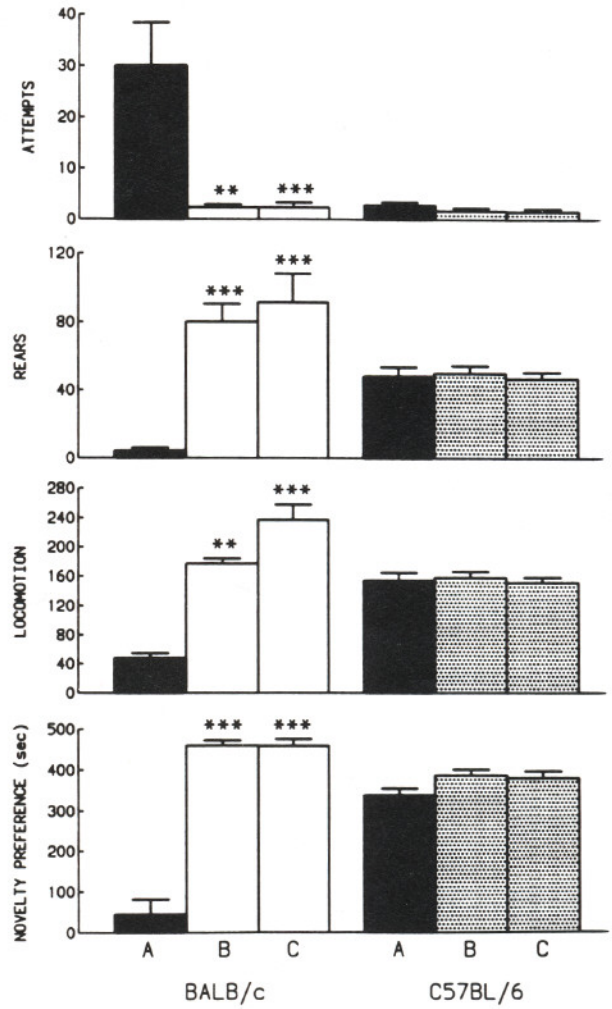

FIG. 1. Behaviour of BALB/c and C57BL/6 mice in the free-exploratory test. Animals were confronted with an empty novel compartment (A), a novel compartment containing fresh sawdust (B) or rubbed with urine of unfamiliar conspecifics (C). The four panels show time spent in the novel compartment (novelty preference), locomotion, rearing behaviour (rears), and attempts at entry into the novel compartment followed by avoidance responses (attempts). Values are means + S.E.M.

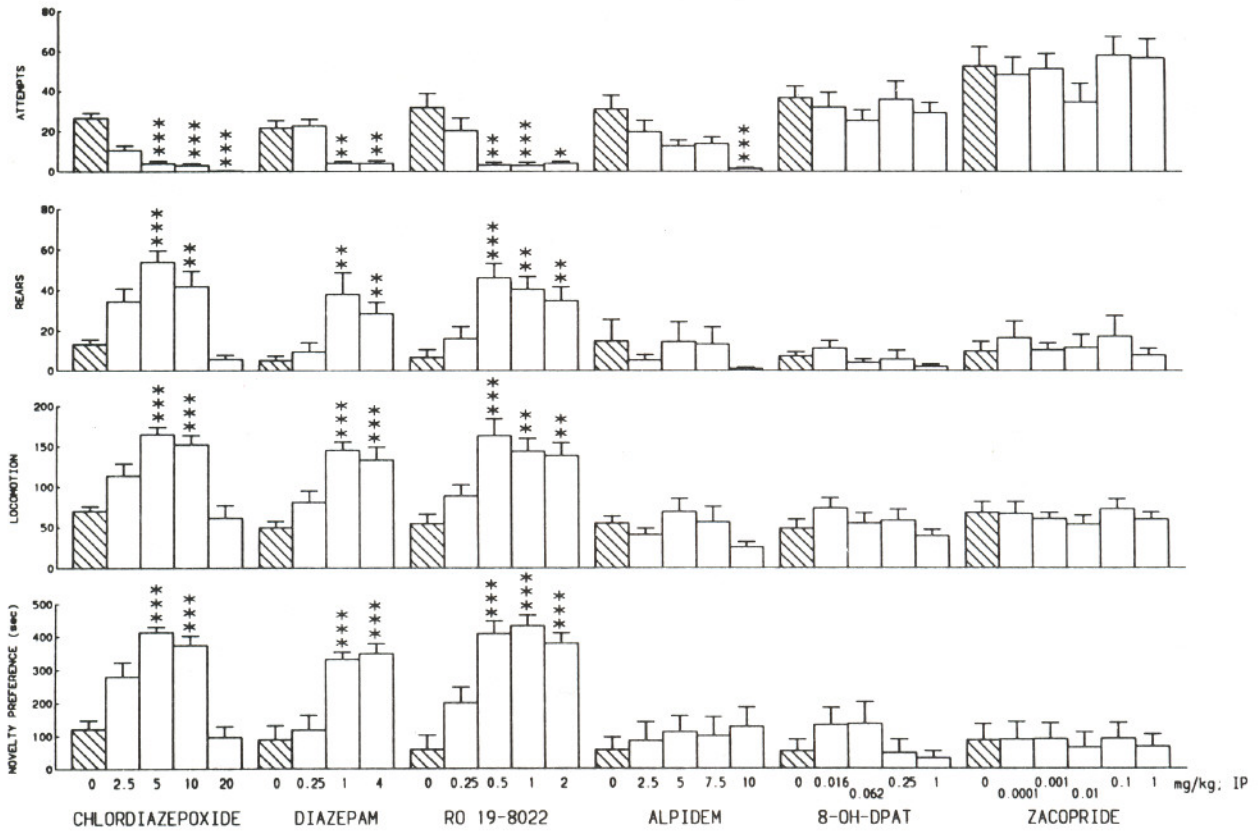

FIG. 2. Effects of chlordiazepoxide, diazepam, Ro 19-8022, alpidem, 8-OH-DPAT and zacopride in BALB/c mice confronted with a free-exploratory test. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$, relative to vehicle treatment (hatched bars). Further details are as in Fig. 1.

Effects of diazepam. Analysis of variance revealed significant differences among groups for time [$F(3,36) = 14.3, p<0.001$], locomotion [$F(3,36) = 13.5, p<0.001$], rears [$KW = 18.2, p<0.001$] and attempts [$KW = 26.4, p<0.001$]. Figure 2 shows that diazepam significantly increased time, locomotion and rears, while it decreased the number of attempts (2 and 4 mg/kg).

Effects of Ro 19-8022. Analysis of variance revealed significant differences among groups for time [$F(4,45) = 17.1, p<0.001$], locomotion [$F(4,45) = 7.8, p<0.001$], rears [$F(4,45) = 7.7, p<0.001$] and attempts [$KW = 24.9, p<0.001$]. Figure 2 shows that Ro 19-8022 displayed a similar pharmacological profile to those of chlordiazepoxide and diazepam, as at some doses it completely reversed the neophobic responses of BALB/c mice.

Effects of alpidem. Analysis of variance revealed no significant differences among groups for time [$F(4,45) = 0.3$], locomotion [$KW = 8.8$] and rears [$KW = 5.3$], while there were significant differences for attempts [$KW = 19.7, p<0.001$]. Figure 2 shows that alpidem, at the highest dose, tended to induce a general decrease in locomotor activities, which may account for the significant decrease in the number of attempts (behavioural sedation).

Effects of 8-OH-DPAT. Analysis of variance did not reveal significant differences among groups for time [$KW = 6.9$], locomotion [$KW = 1.2$] or for attempts [$F(4,45) = 0.5$], but did for rears [$KW = 11.4, p<0.02$]. Figure 2 shows that 8-OH-DPAT did not significantly modify the behaviour of treated mice when compared with controls.

Effects of zacopride. Analysis of variance did not reveal significant differences between groups either for time [$F(5,54) = 0.1$], locomotion [$F(5,54) = 0.4$], rears [$KW = 1.1$] or for attempts [$F(5,54) = 0.9$] (Fig. 2).

Experiment 2: C57BL/6

Effects of chlordiazepoxide. Analysis of variance revealed significant differences among groups for loco-

motion [F(4,45) = 15.1, $p < 0.001$] and rears [KW = 23.9, $p < 0.001$], but not for time (KW = 4) or for attempts [F(4,45) = 1.1]. Figure 3 shows that chlordiazepoxide significantly increased locomotion at 5 and 10 mg/kg, while it decreased rears at the highest dose (20 mg/kg).

Effects of diazepam. Analysis of variance revealed significant differences among groups for time [KW = 9.2, $p < 0.02$] and rears [KW = 9.6, $p < 0.02$], but not for locomotion [KW = 3.2] or attempts [KW = 5.6]. Figure 3 shows that individual statistical comparisons did not show any significant differences from control values with any individual dose of diazepam.

Effects of Ro 19-8022. Analysis of variance did not reveal any significant differences among groups for time [F(4,45) = 1.1], locomotion [F(4,45) = 2.5], rears [F(4,45) = 0.8] or attempts [F(4,45) = 0.7].

Effects of alpidem. Analysis of variance did not reveal any significant differences among groups for time [F(4,45) = 0.3], locomotion [F(4,45) = 0.1], rears [F(4,45) = 0.2] or attempts [F(4,45) = 2.2].

Effects of 8-OH-DPAT. Analysis of variance did not reveal significant differences among groups for time [F(4,45) = 0.7], locomotion [F(4,45) = 0.3] or for attempts [F(4,45) = 0.5]. There was a statistically significant effect on rears [F(4,45) = 7.4, $p < 0.001$], which were decreased by 8-OH-DPAT at 1 mg/kg.

Effects of zacopride. Analysis of variance did not reveal significant differences between groups either for time [F(5,54) = 0.4], locomotion [F(5,54) = 0.3], rears [F(5,54) = 1.0] or attempts [KW = 3.8] (Fig. 3).

DISCUSSION

The present results showed that BALB/c mice, confronted simultaneously with familiar and novel places in a free-exploratory test, exhibited a marked preference for the familiar compartment while making a considerable number of attempts at entry into the novel compartment. This behaviour strongly contrasted with that of Swiss (Misslin and Ropartz, 1981) or C57BL/6 (present results) mice confronted with the same experimental situation: the latter animals showed a small preference for the novel compart-

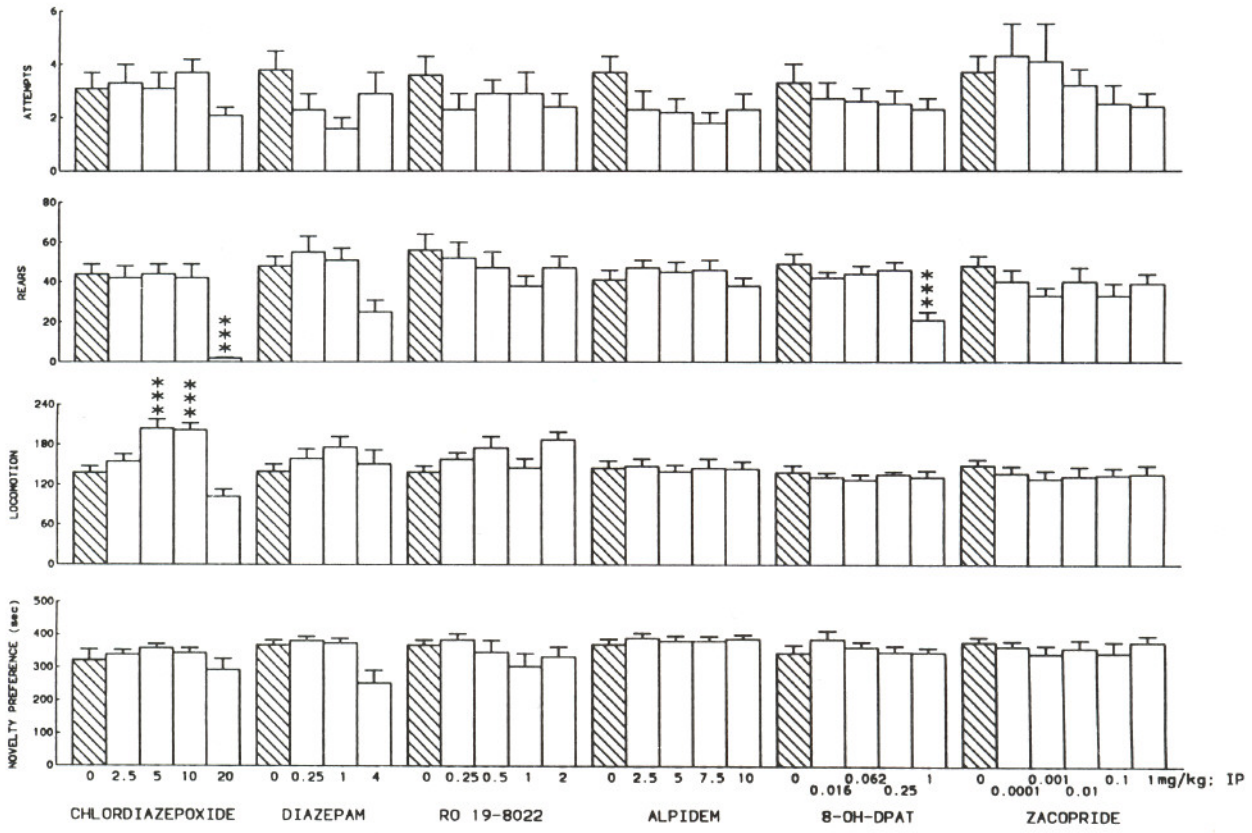

FIG. 3. Effects of chlordiazepoxide, diazepam, Ro 19-8022, alpidem, 8-OH-DPAT and zacopride in C57BL/6 mice confronted with a free-exploratory test. *** $p < 0.001$, relative to vehicle-treated animals (hatched bars). Further details are as in Fig. 1.

ment. When confronted with the novel compartments, BALB/c mice exhibited so many attempts at entry that the overall level of their locomotion and rears was lowered in comparison with that of C57BL/6 mice. Indeed, there was a significant negative correlation between the number of attempts in BALB/c mice and the other behavioural parameters. Since the free-exploratory procedure has been found to be devoid of intrinsic stressful elements (Misslin *et al.*, 1982; Misslin and Cigrang, 1986), this test provides an effective way of measuring specific neophobic responses in BALB/c mice. The preference of these mice for the familiar compartment was completely reversed by adding fresh sawdust or urine of unfamiliar conspecifics to the novel compartment. This demonstrates that unfamiliarity was the determining factor in revealing neophobia in BALB/c mice. It is to be noted that neither fresh sawdust nor the urine of conspecifics modified the preference of C57BL/6 mice for the novel compartment.

The benzodiazepine full agonists, chlordiazepoxide and diazepam, reversed the preference of BALB/c mice: drug-treated animals exhibited a preference for the novel compartment and very few avoidance responses. The quinolizinone, Ro 19-8022, which binds to benzodiazepine receptors, was also found to be a potent drug in counteracting the spontaneous propensity of BALB/c mice to avoid novelty. This compound was recently described as having strong anticonflict effects in mice and rats (Jenck *et al.*, 1992). In contrast to the neophobia-reducing effects of these three drugs, the imidazopyridine, alpidem, failed to antagonize significantly the preference of BALB/c mice for the familiar compartment. Although alpidem has been reported to possess anxiolytic-like effects in several animal models of fear (e.g. Vogel conflict test, marble-burying test), its pharmacological profile appeared to be substantially different from that of benzodiazepines, as the range of experimental situations in which it showed anxiolysis was not so large as that of diazepam or chlordiazepoxide (Zivkovic *et al.*, 1990; Sanger *et al.*, 1993). Moreover, in rats trained to discriminate chlordiazepoxide from saline, alpidem failed to produce a benzodiazepine-like interoceptive stimulus. In addition, the binding profile of alpidem contrasts with that of benzodiazepines as it selectively binds to the ω_1 (BZ_1) and ω_3 (peripheral-type) receptors, while benzodiazepines show no selective affinity for ω_1 or ω_2 receptor subtypes (Zivkovic *et al.*, 1990). Zivkovic *et al.* (1990) suggested that anxiolytic-like effects of alpidem can be related to its selectivity for the ω_1 subtype since these effects were antagonized by flumazenil, which showed no affinity for the ω_3 receptor. Thus, it is possible that the receptor selectivity of alpidem may account for its inability to counteract the neophobia of BALB/c mice. In contrast, it has been shown that Ro 19-8022, which displayed a pharmacological profile very similar to that of chlordiazepoxide or diazepam in the present paradigm, did not bind selectively to heterogeneous benzodiazepine recognition sites (Jenck *et al.*, 1992). In addition, it should be noted that, at the highest doses tested, chlordiazepoxide, as well as alpidem, sharply decreased the number of attempts. This action is probably related to their sedative properties as locomotor activity and rears also tended to be decreased, although these effects were not statistically significant.

It is now well documented that some central 5-HT receptors are implicated in modulating anxiety states. In some animal models of anxiety, 5-HT_{1A} receptor agonists, such as 8-OH-DPAT, buspirone, gepirone or ipsapirone (for review, see Treit, 1991) and 5-HT_3 receptor antagonists such as MDL72222, ondansetron and zacopride (for review, see Costall and Naylor, 1991) have been shown to possess an anxiolytic profile similar to that of diazepam, although these effects are often more variable than those of classical anxiolytics. Thus, 8-OH-DPAT has been found to possess anxiolytic-like effects especially in situations involving unconditioned behaviour such as the light/dark choice procedure in mice (e.g. Misslin *et al.*, 1990), the social interaction test in rats (e.g. Higgins *et al.*, 1992), the open-field test in rats (e.g. Carli *et al.*, 1989), the staircase test in mice (Boaventura *et al.*, 1986) or the elevated plus-maze in rats (e.g. Dunn *et al.*, 1989). However, conflicting results in the elevated plus-maze in rats have been reported for this compound. Indeed, some studies reported anxiogenic-like effects (e.g. Critchley and Handley, 1987) while others found no evidence for anxiolytic- or anxiogenic-like actions (File *et al.*, 1987; Moser *et al.*, 1990). These sources of interlaboratory variability on drug effects may be explained by the different experimental conditions used, such as level of illumination or daytime observation period (Griebel *et al.*, 1993). Interestingly, an alternative explanation for the inconsistent effects of drugs acting at 5-HT receptors is that anxiolysis is evident only in particular experimental conditions, i.e. in models of fear-motivated and constraining situations (Carli and Samanin, 1988; Higgins *et al.*, 1992). For example, Carli and Samanin (1988) found that 8-OH-DPAT displayed anxiolytic-like effects only when the animals had previously been subjected to immobilization stress, while Higgins *et al.* (1992) observed that 8-OH-DPAT failed to modify the behaviour of rats confronted with the social interaction test under low illumination conditions and familiarity with the test arena.

It is now well established that 5-HT_3 receptor antagonists, including zacopride, are able to reduce unconditioned anxious responses of various species, such as mice (e.g. Costall *et al.*, 1987), rats (e.g. Piper *et al.*, 1988), gerbils (e.g. Cutler, 1990), marmosets (e.g. Tyers *et al.*, 1987) and cynomolgus monkeys (e.g. Jones *et al.*, 1987). The present data, which show that 8-OH-DPAT and zacopride did not counteract the preference of BALB/c mice for the familiar

compartment, could be consistent with the idea that these drugs are specifically effective in situations which assess "state" anxiety (Lister, 1990). Since the free-exploratory test has been found to be devoid of constraining and stressful components (Misslin *et al.*, 1982; Misslin and Cigrang, 1986), it can be suggested that neophobia in BALB/c mice is related to what Lister (1990) called "trait" anxiety, which appears to be insensitive to 5-HT receptor-modulating drugs.

Finally, since C57BL/6 mice did not exhibit defensive responses toward novelty, but on the contrary were found to show a significant preference for the novel compartment, one could suppose that anxiolytics would not substantially modify their behaviour. Indeed, our findings indicate that drugs which reversed the preference of BALB/c mice for familiar places, did not significantly affect the specific responses of C57BL/6 mice toward novelty. Nevertheless, it must be noted that chlordiazepoxide increased locomotion at 5 and 10 mg/kg. This effect is probably related to the so-called disinhibitory action which has sometimes been observed in non-stressful situations (File and Hyde, 1979). Moreover, at the highest doses, chlordiazepoxide as well as 8-OH-DPAT markedly decreased rears, which probably can be considered as the onset of behavioural depression. This latter effect confirms the depressant properties we recently found with 8-OH-DPAT in Swiss mice confronted with the same experimental paradigm (Misslin *et al.*, 1990).

In summary, chlordiazepoxide, diazepam and Ro 19-8022, which interact in a non-specific manner with benzodiazepine receptor subtypes, completely reversed neophobia in BALB/c mice confronted with a free-exploratory paradigm, while 8-OH-DPAT and zacopride which act at 5-HT receptors, and alpidem which binds selectively to certain benzodiazepine receptor subtypes, were unable to modify the neophobic behaviour of BALB/c mice. The free-exploratory situation may be proposed as a specific animal model of "trait" anxiety. The present results suggest that the classical anxiolytics, which are effective in animal models of "state" anxiety, are not equally effective in reducing neophobia in BALB/c mice.

REFERENCES

Archer J (1973) Test for emotionality in rats and mice: a review. *Animal Behavior*, **21**, 205-235.

Blanchard RJ, Kelley MJ and Blanchard DC (1974) Defensive reactions and exploratory behavior in rats. *Journal of Comparative and Physiological Psychology*, **87**, 1129-1133.

Boaventura AM, Blaquiere B, Palou AM and Oberlander C (1986) Possible involvement of 5-HT_{1A} sites in the anxiolytic response to diazepam. *Neuroscience Letters*, **26**, S278 (suppl).

Carli M and Samanin R (1988) Potential anxiolytic properties of 8-hydroxy-2-(di-*N*-propylamino)tetralin, a selective serotonin$_{1A}$ receptor agonist. *Psychopharmacology*, **94**, 84-91.

Carli M, Prontera C and Samanin R (1989) Effect of 5-HT_{1A} agonists on stress-induced deficit in open field locomotor activity of rats, evidence that this model identifies anxiolytic-like activity. *Neuropharmacology*, **28**, 471-476.

Costall B and Naylor RJ (1991) Anxiolytic effects of 5-HT_3 antagonists in animals. In: *5-HT_{1A} Agonists, 5-HT_3 Antagonists and Benzodiazepines: Their Comparative Behavioural Pharmacology* (Eds RJ Rodgers and SJ Cooper), pp. 133-157. John Wiley, Chichester.

Costall B, Domeney AM, Kelly ME, Naylor RJ and Tyers MB (1987) The behavioural consequences of treatment with selective 5-HT_3 receptor antagonists. *British Journal of Pharmacology*, **92**, 657P (suppl).

Costall B, Jones BJ, Kelly ME, Naylor RJ and Tomkins DM (1989) Exploration of mice in a black and white test box: validation as a model of anxiety. *Pharmacology Biochemistry and Behavior*, **32**, 777-785.

Crawley JN and Goodwin FK (1980) Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacology Biochemistry and Behavior*, **13**, 167-170.

Critchley MAE and Handley SL (1987) Effects in the X-maze model of agents acting at 5-HT_1 and 5-HT_2 receptors. *Psychopharmacology*, **93**, 502-506.

Cutler MG (1990) Behavioural effects in gerbils of the 5-HT_3 receptor antagonists, BRL 43694 and ICS 205-930, under circumstances of high and low light intensity. *Neuropharmacology*, **29**, 515-520.

Dunn RW, Corbett R and Fielding S (1989) Effects of 5-HT_{1A} receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze. *European Journal of Pharmacology*, **169**, 1-10.

File SE and Hyde JRG (1979) A test of anxiety that distinguishes between the action of benzodiazepines and those of other minor tranquilizers and of stimulants. *Pharmacology Biochemistry and Behavior*, **11**, 65-69.

File SE, Johnston AL and Pellow S (1987) Effects of compounds acting at CNS 5-hydroxytryptamine systems on anxiety in the rat. *British Journal of Pharmacology*, **90**, 265P.

Griebel G, Moreau J-L, Jenck F, Martin JR and Misslin R (1993) Some critical determinants of the behavior of rats in the elevated plus-maze. *Behavioral Processes*, **29**, 37-48.

Higgins GA, Jones BJ and Oakley NR (1992) Effect of 5-HT_{1A} receptor agonists in two models of anxiety after dorsal raphe injection. *Psychopharmacology*, **106**, 261-267.

Hughes RN (1968) Behaviour of male and female rats with a free choice of two environments differing in novelty. *Animal Behavior*, **16**, 92-96.

Jenck F, Moreau JL, Bonetti EP, Martin JR and Haefely WE (1992) Ro 19-8022, a non benzodiazepine partial agonist at benzodiazepine receptors: neuropharmacological profile of a potent anxiolytic. *Journal of Pharmacology and Experimental Therapeutics*, **262**, 1121-1127.

Jones BJ, Oakley NR and Tyers MB (1987) The anxiolytic activity of GR38032F, a 5-HT_3 receptor antagonist, in the rat and cynomolgus monkey. *British Journal of Pharmacology*, **90**, 88P (suppl.).

Lister RG (1990) Ethologically-based animal models of anxiety disorders. *Pharmacology and Therapeutics*, **46**, 321-340.

Misslin R (1983) Contribution neuroéthologique à l'étude des conduites néotiques chez la souris. Thèse d'Etat, Université L. Pasteur de Strasbourg.

Misslin R and Cigrang M (1986) Does neophobia necessarily imply fear or anxiety? *Behavioural Processes*, **12**, 45-50.

Misslin R and Ropartz P (1981) Effects of methamphetamine on novelty-seeking behaviour by mice. *Psychopharmacology*, **75**, 39-43.

Misslin R, Herzog F, Koch B and Ropartz P (1982) Effects of isolation, handling and novelty on the pituitary-adrenal response in the mouse. *Psychoneuroendocrinology*, **7**, 217-221.

Misslin R, Belzung C and Vogel E (1989) Behavioural validation of a light/dark choice procedure for testing anti-anxiety agents. *Behavioural Processes*, **8**, 119-132.

Misslin R, Griebel G, Saffroy-Spittler M and Vogel E (1990) Anxiolytic and sedative effects of 5-HT_{1A} ligands, 8-OH-DPAT and MDL 73005EF, in mice. *NeuroReport*, **1**, 267-270.

Moser PC, Tricklebank MD, Middlemiss DN, Mir AK, Hibert MF and Fozard JR (1990) Characterization of MDL 73005EF as a 5-HT_{1A} selective ligand and its effects in animal models of anxiety, comparison with buspirone, 8-OH-DPAT and diazepam. *British Journal of Pharmacology*, **99**, 343-349.

Oliverio A, Eleftheriou BE and Bailey DW (1973) A gene influencing active avoidance performance in mice. *Physiology and Behavior*, **11**, 497-501.

Peeler DF and Nowakowski RS (1987) Genetic factors and the measurement of exploratory activity. *Behavioral and Neural Biology*, **48**, 90-103.

Pellow S, Chopin P, File SE and Briley M (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, **14**, 149-167.

Piper DC, Upton N, Thomas D and Nicholson J (1988) The effects of 5-HT_3 receptor antagonists BRL 43694 and GR 38032F in animal behavioural models of anxiety. *British Journal of Pharmacology*, **94**, 314P (suppl).

Russel PA (1973) Relationships between exploratory behavior and fear: a review. *British Journal of Psychology*, **64**, 417-433.

Sanger DJ (1991) Animal models of anxiety and the screening and development of novel anxiolytic drugs. In: *Neuromethods, Vol. 19, Animal Models in Psychiatry, II* (Eds AA Boulton, GB Baker and M Martin-Iverson), pp. 147-198. Humana Press, Clifton, NJ.

Sanger DJ, Perrault GH, Morel E, Joly D and Zivkovic B (1993) The pharmacological profile of alpidem. In: *Imidazopyridines in Anxiety Disorders*, in press. Raven Press, New York.

Thiébot MH, Soubrié P, Simon P and Boissier JR (1973) Dissociation de deux composantes du comportement chez le rat sons l'effet de psychotropes. Application à l'étude des anxiolytiques. *Psychopharmacologia*, **31**, 77-90.

Treit D (1991) Anxiolytic effects of benzodiazepines and 5-HT_{1A} agonists: animal models. In: *5-HT_{1A} Agonists, 5-HT_3 Antagonists and Benzodiazepines: Their Comparative Behavioural Pharmacology* (Eds RJ Rodgers and SJ Cooper), pp. 107-131. John Wiley, Chichester.

Tyers MB, Costall B, Domeney A, Jones BJ, Kelly ME, Naylor RJ and Oakley NR (1987) The anxiolytic activities of $5HT_3$ antagonists in laboratory animals. *Neuroscience Letters*, **29**, S68 (suppl).

Walsh RN and Cummins RA (1976) The open-field test: a critical review. *Psychological Bulletin*, **83**, 482-504.

Welker WI (1957) "Free" versus "forced" exploration of a novel situation by rats. *Psychological Reports*, **3**, 95-108.

Zivkovic B, Morel E, Joly D, Perrault G, Sanger DJ and Lloyd K (1990) Pharmacological and behavioral profile of alpidem as an anxiolytic. *Pharmacopsychiatry*, **23**, 108-113.

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