Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 459 (2003) 221–230
www.elsevier.com/locate/ejphar

Prior exposure to the elevated plus-maze sensitizes mice to the acute behavioral effects of fluoxetine and phenelzine

Andrew Holmes*, R. John Rodgers

Behavioural Pharmacology Laboratory, School of Psychology, University of Leeds, Leeds LS2 9JT, United Kingdom

Received 18 July 2002; received in revised form 3 December 2002; accepted 6 December 2002

Abstract

A single undrugged experience of the elevated plus-maze modifies future drug responses in the test. The present study investigated the effects of maze-experience on the acute behavioral effects of the monoamine oxidase inhibitor phenelzine and the serotonin reuptake inhibitor fluoxetine. Phenelzine (2.5–12.5 mg/kg) had no clear effect on plus-maze behavior in test-naive Swiss Webster mice, but dose-dependently increased anxiety-like behavior in maze-experienced subjects. Similarly, fluoxetine (5–20 mg/kg) produced non-significant trends for increased anxiety-like behavior in maze-naive mice, but significantly and dose-dependently increased anxiety-like behavior and suppressed locomotor activity in maze-experienced mice. The anxiogenic effects of the benzodiazepine receptor inverse agonist *N*-methyl-β-carboline-3-carboxamide (FG 7142) (20 mg/kg) was abolished by prior test experience, suggesting an alteration in γ-aminobutyric acid (GABA)/benzodiazepine receptor function with maze-experience. However, the benzodiazepine receptor antagonist flumazenil (5–20 mg/kg) produced a silent profile regardless of maze-experience. Present findings provide further evidence demonstrating that prior test history is a critical consideration in mouse studies of anxiety-related behavior.

Keywords: Elevated plus-maze; Test experience; Anxiety; Antidepressant; Benzodiazepine; (Mouse)

1. Introduction

Transgenic and gene knockout mice are costly to initially generate, while extensive breeding is required in order to produce congenic mutant lines for sound behavioral studies. For these reasons, it has become common practice to economize the use of subjects by employing behavioral test batteries in which mice are subject to multiple assays (Crawley and Paylor, 1997; Rogers et al., 1997; McIlwain et al., 2001). Furthermore, for experiments involving pharmacological challenge, gene mutant mice may be subject to multiple trials on a given test under each treatment condition. In this context, there is a wealth of literature demonstrating that in models of anxiety-like behavior, test experience can alter subsequent responses within the same test. Thus, a single undrugged test exposure markedly alters the behavioral profile of various drugs on subsequent testing in the elevated plus-maze (for reviews, see Holmes and Rodgers, 1999; File, 2001), light ↔ dark exploration (Holmes and Rodgers, 2001), predator-odor-exposure (McGregor and Dielenberg, 1999), and four-plate (Hascoet et al., 1997) tests for anxiety-like behavior.

In the elevated plus-maze, previous research has demonstrated that changes occurring with test-experience involve rapid, amygdala-mediated learning during the initial stages of the first plus-maze exposure (Rodgers et al., 1996; File et al., 1998; Holmes and Rodgers, 1998). The effects of maze-experience on drug responses are not explained by a habituation to the anxiety-provoking aspects of the test, as stress hormone responses to plus-maze exposure do not decrease with repeated testing (File et al., 1994; Holmes et al., 1998). Instead, principal components factor analysis of behavioral scores from repeated plus-maze exposures suggests that a qualitative shift in the nature of the anxiety response underlies changes in pharmacological responses with retesting (File, 1993; Holmes and Rodgers, 1998).

Pharmacological characterization of the effects of plus-maze retesting in mice have largely been restricted to the effects of prior maze-experience on the anxiolytic efficacy of benzodiazepines. Studies in both rats and mice have

* Corresponding author. Section on Behavioral Genomics, National Institute of Mental Health, Building 10, Room 4D11, Bethesda, MD 20892-1375, USA. Tel.: +1-301-496-4838; fax: +1-301-480-1164.
E-mail address: aholmes@intra.nimh.nih.gov (A. Holmes).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(02)02874-1

shown that prior maze-experience reduces or abolishes the anxiolytic efficacy of benzodiazepine receptor agonists such as chlordiazepoxide, midazolam and diazepam (Lister, 1987; File, 1990; File et al., 1990; Rodgers et al., 1992; File and Zangrossi, 1993; Rodgers and Shepherd, 1993; Fernandes and File, 1996; Holmes and Rodgers, 1999). However, relatively little attention has been paid to the effects of plus-maze experience on other classes of anxiolytic. The primary purpose of the present study was to test whether maze experience alters the behavioral profile of anxiolytics that target central monoamine systems.

Phenelzine is a monoamine oxidase inhibitor that causes increases in synaptic levels of serotonin, norepinephrine and dopamine by blocking the activity of the monoamine degradation enzyme monoamine oxidase. Phenelzine is clinically effective against depression, as well as a broad spectrum of anxiety disorders, including Panic Disorder, Post-traumatic Stress Disorder and Social Phobia (Liebowitz et al., 1990; Ballenger, 1999). Serotonin reuptake inhibitors are among the most widely used drugs for mood and anxiety disorders (Stokes and Holtz, 1997; Stein and Berk, 2000). Drugs of this class, such as fluoxetine, inhibit the action of the serotonin transporter and, thereby, increase serotonin availability (Blakely et al., 1991). In the present study, we have examined the acute effects of phenelzine and fluoxetine in maze-naive and maze-experienced mice. The therapeutic effects of phenelzine and fluoxetine have been associated with their ability to enhance γ-aminobutyric acid (GABA) function in the brain (Paslawski et al., 1996; Uzunova et al., 1998; Tunnicliff et al., 1999; Belzung et al., 2001; Matsubara et al., 2000; Tanay et al., 2001; Parent et al., 2002). Therefore, to further investigate changes in GABA function following plus-maze experience, and to extend evidence of a loss of benzodiazepine receptor agonist activity with retesting in the plus-maze, we have also assessed the influence of maze-experience on the effects of a benzodiazepine receptor partial inverse agonist (*N*-methyl-β-carboline-3-carboxamide; FG 7142) and a benzodiazepine receptor antagonist (flumazenil).

2. Material and methods

2.1. Subjects

Subjects were adult male Swiss-Webster mice (Bantin and Kingman, Hull, UK), 11–12 weeks old at the time of testing. They were housed in groups of 10 (cage size: 45 × 28 × 13 cm), under a 12-h reversed light cycle (lights off: 07:00 h), in a temperature (21 ± 1 °C) and humidity (50 ± 5%) controlled environment. Food and drinking water were freely available except during brief test periods. All subjects were experimentally naive at the start of the study, and had been previously handled only for the purpose of routine husbandry.

2.2. Drugs

Fluoxetine hydrochloride and phenelzine sulphate were both obtained from Sigma, UK; flumazenil was obtained from Hoffman-la-Roche, Basel; *N*-methyl-β-carboline-3-carboxamide (FG 7142) was obtained from Research Biochemicals, USA. All drugs were dissolved in physiological saline (FG 7142 and flumazenil with two drops of Tween 80) and administered intraperitoneally in a volume of 10 ml/kg body weight 30 min prior to testing.

2.3. Apparatus

The elevated plus-maze (Handley and Mithani, 1984; Pellow et al., 1985; Holmes, 2001) comprised two open arms (30 × 5 × 0.25 cm) and two closed arms (30 × 5 × 15 cm) that extend from a common central platform (5 × 5 cm) (Lister, 1987). The apparatus was constructed from Plexiglas (black floor, clear walls), elevated to a height of 60 cm above floor level and illuminated under dim red light (4 × 100 W, indirect) (Holmes et al., 1998). A slight raised edge (0.25 cm) around the perimeter of the open arms prevented mice falling from the maze.

2.4. General procedure

Subjects were transported to the dimly illuminated laboratory during the dark phase of the light/dark cycle and left undisturbed for 1 h prior to testing. Subjects were equally divided into two experimental groups; mice that were test-naive and mice that had been pre-exposed (undrugged) to the elevated plus-maze 24 h earlier. Mice were individually placed in the central square of the elevated plus-maze (facing an open arm) and allowed to freely explore the apparatus for 5 min in an order counterbalanced for drug treatment and test-experience. Between subjects, the apparatus was thoroughly cleaned with wet and dry cloths. A camera positioned above at an angle of ~ 50° to the maze recorded test sessions for later analysis. To avoid distractions, the experimenter monitored sessions in an adjacent room via videolink.

2.5. Behavioral measures

Videotapes were scored blind by a highly trained observer (intra-rater reliability ≥ 0.9) using ethological software (“Hindsight” v.1.4, Scientific Programming Services, Wokingham, UK). Behavioral parameters comprised both conventional spatiotemporal and ethological measures (Rodgers and Cole, 1994; Rodgers et al., 1999; Holmes et al., 2000). Conventional measures were the total number of arm entries (arm entry = all four paws into an arm), the number of closed entries, the percentage of arm entries into the open arms and the percentage of the session spent in open arms. Ethological measures comprised frequency

scores for stretched-attend postures (exploratory posture in which the body is stretched forward then retracted to the original position without any forward locomotion) and head-dipping (exploratory movement of head/shoulders over the side of the maze).

2.6. Experiment 1: effects of the monoamine oxidase inhibitor, phenelzine

The effects of phenelzine were assessed in 96 mice. Within maze-naive and maze-experienced groups, mice were randomly allocated to either vehicle, 0.5, 2.5 or 12.5 mg/kg phenelzine ($n = 12$). Doses were chosen on the basis of previous studies on anxiety-like behavior in mice (e.g., Griebel et al., 1998; Rupniak et al., 2000).

2.7. Experiment 2: effects of the serotonin reuptake inhibitor, fluoxetine

The effects of fluoxetine were assessed in 96 mice. Within maze-naive and maze-experienced groups, mice were randomly allocated to either vehicle, 5, 10 or 20 mg/kg fluoxetine ($n = 11–13$). Doses were chosen on the basis of previous studies on anxiety-like behavior in mice (e.g., Griebel et al., 1997; Belzung et al., 2001).

2.8. Experiment 3: effects of the benzodiazepine partial inverse agonist, FG 71242

The effects of FG 7142 were assessed in 48 mice. Within maze-naive and maze-experienced groups, mice were

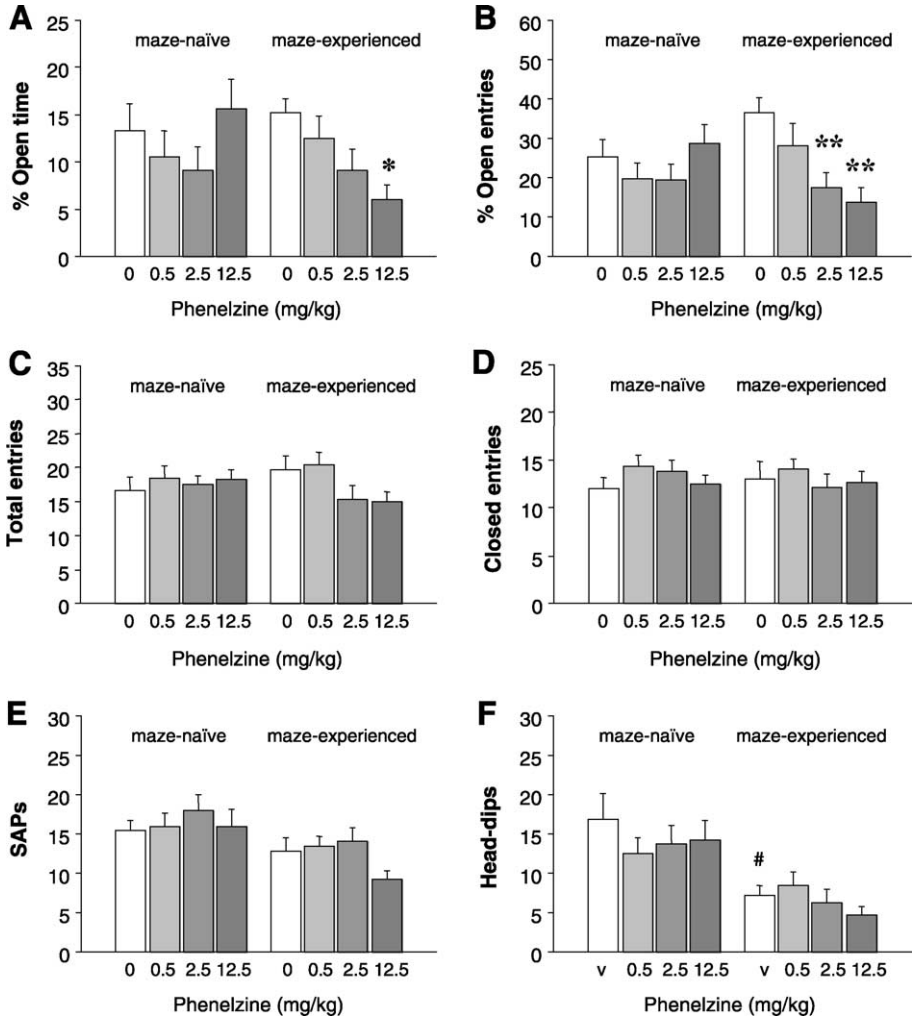

Fig. 1. The monoamine oxidase A inhibitor, phenelzine, produced anxiogenic-like effects in maze-experienced mice, but no significant effects in maze-naive mice. In mice exposed to the plus-maze, 24 h prior to testing, phenelzine dose-dependently reduced % open time (A) and % open entries (B), but had no effect on total entries (C), closed entries (D), stretched-attend postures (E) or head-dips (F). In maze-naive mice, phenelzine produced no significant effect on plus-maze behavior. Maze-experience per se had minimal effect on baseline plus-maze behavior; head-dips were significantly lower in vehicle-treated, maze-experienced mice than maze-naive counterparts. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. vehicle (0). $^{\#}P < 0.05$ vs. (vehicle) maze-naive ($n = 12$). Data for all figures are means ± SEM.

randomly allocated to either vehicle or 20 mg/kg FG 7142 ($n = 12$). This dose was chosen on the basis of previous studies in the rat and mouse plus-maze (e.g., File and Zangrossi, 1993; Rodgers et al., 1995).

2.9. Experiment 4: effects of the benzodiazepine antagonist, flumazenil

The effects of flumazenil were assessed in 85 mice. Within maze-naive and maze-experienced groups, mice were randomly allocated to vehicle, 5, 10, or 20 mg/kg flumazenil ($n = 10–11$). Doses were chosen on the basis of previous studies in the rat and mouse plus-maze (e.g., File et al., 1998; Dalvi and Rodgers, 1999).

2.10. Statistical analysis

Maze-experience and drug effects were analyzed using two-factor between-groups analyses of variance (ANOVA) (maze-experience × drug) and were followed by Newman–Keuls post-hoc analysis conducted separately for maze-naive and maze-experienced groups. P values were accepted as significant at $P < 0.05$.

2.11. Ethics

The research described in this paper was licensed by the Home Office under the Animals (Scientific Procedures) Act 1986.

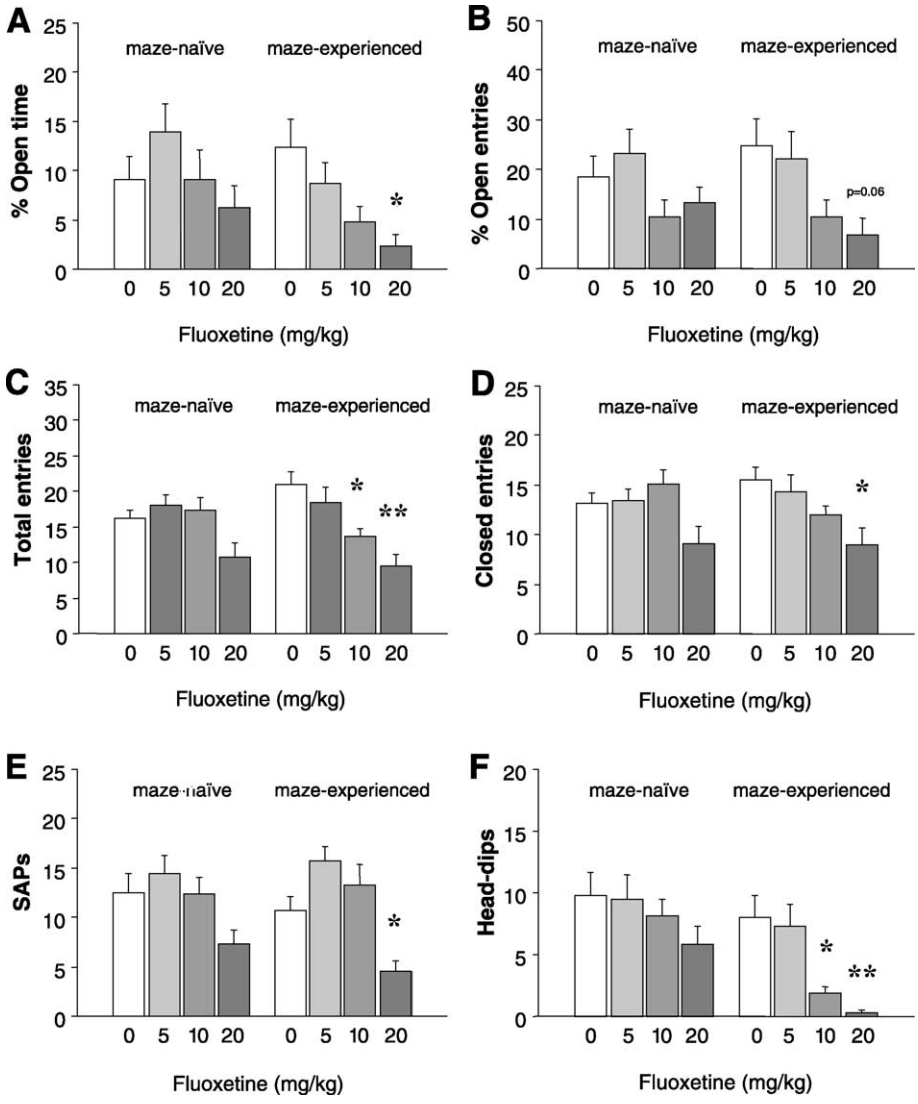

Fig. 2. The serotonin reuptake inhibitor, fluoxetine, produced anxiogenic-like and locomotor-suppressant effects in maze-experienced mice, but no significant effects in maze-naive mice. In mice exposed to the plus-maze, 24 h prior to testing, fluoxetine dose-dependently reduced % open time (A), but had no significant effects on % open entries (B), total entries (C), closed entries (D), stretched-attend postures (E), or head-dips (F). In maze-naive mice, fluoxetine produced no significant effect on plus-maze behavior. Maze-experience per se had minimal effect on baseline plus-maze behavior. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. vehicle (0). ($n = 11–13$).

3. Results

3.1. Experiment 1: effects of phenelzine

ANOVA found significant interactions between maze-experience and the effects of phenelzine for % open time ($F[3,88]=2.71$, $P<0.05$) and % open entries ($F[3,88]=3.85$, $P<0.01$). There was also a significant main effect of phenelzine on % open entries ($F[3,88]=3.85$, $P<0.01$). Post-hoc analyses showed that, in maze-experienced mice, 2.5 mg/kg phenelzine significantly reduced % open time, while 12.5 mg/kg phenelzine significantly reduced both % open time and % open entries (Fig. 1A,B). In maze-naive mice, phenelzine did not significantly affect measures of open arm exploration at any dose (Fig. 1A,B). There were no effects of phenelzine on total entries, closed entries, stretched-attend postures, or head-dips, regardless of maze-experience (Fig. 1C–F). There was a significant main effect of maze-experience on stretched-attend postures ($F[1,88]=11.17$, $P<0.001$) and head-dips ($F[1,88]=26.53$, $P<0.001$). Head-dips were significantly lower in vehicle-treated maze-experienced mice, as compared to vehicle-treated maze-naive mice (Fig. 1F), while stretched-attend postures were not significantly different between these two groups.

3.2. Experiment 2: effects of fluoxetine

ANOVA found no significant interactions between maze-experience and the effects of fluoxetine. There was a significant main effect of fluoxetine on % open time ($F[3,85]=3.85$, $P<0.01$), % open entries ($F[3,85]=5.28$, $P<0.001$), total entries ($F[3,85]=11.06$, $P<0.001$), closed entries ($F[3,85]=6.11$, $P<0.001$), stretched-attend postures ($F[3,85]=11.67$, $P<0.001$), and head-dips ($F[3,85]=7.03$, $P<0.001$). Post-hoc analysis showed that, in maze-experienced mice, 20 mg/kg fluoxetine significantly reduced

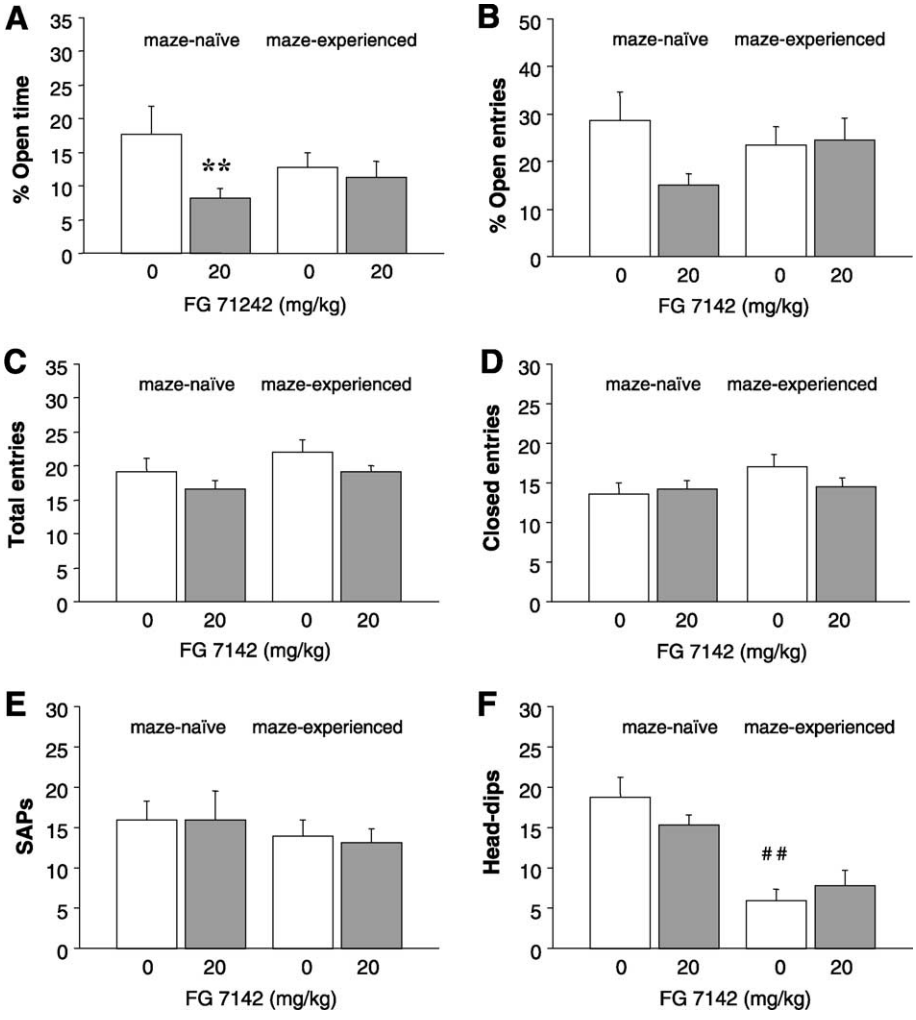

Fig. 3. The benzodiazepine partial inverse agonist, FG 7142, produced anxiogenic-like effects in maze-naive, but not maze-experienced, mice. In maze-naive mice, FG 7142 produced a significant reduction in % open time (A) and a non-significant trend for reduced % open entries (B), had no effect on total entries (C), closed entries (D), stretched-attend postures (E) or head-dips (F). In maze-experienced mice, FG 7142 produced no significant effect on plus-maze behavior. Maze-experience per se had minimal effect on baseline plus-maze behavior; head-dips were significantly lower in vehicle-treated, maze-experienced mice than maze-naive counterparts. $^{**}P<0.01$ vs. vehicle (0). $^{\#\#}P<0.01$ vs. (vehicle) maze-naive. ($n=12$).

% open time, total entries, closed entries, stretched-attend postures, and head-dips (% open entries approached significance at $P=0.059$), while 10 mg/kg fluoxetine significantly reduced total entries and head-dips (Fig. 2A–F). In maze-naive mice, fluoxetine was without significant effect on any measure at any dose (Fig. 2A–F). There was a significant main effect of maze-experience on head-dips ($F[1,83]=14.40$, $P<0.001$). Head-dips were significantly lower in vehicle-treated maze-experienced mice, as compared to vehicle-treated maze-naive mice (Fig. 2F).

3.3. Experiment 3: effects of FG 71242

ANOVA found no significant interactions between maze-experience and the effects of FG 7142. There was a significant main effect of FG 7142 on % open time ($F[1,44]=4.07$, $P<0.05$). FG 7142 significantly reduced % open time in maze-naive, but not maze-experienced, mice (Fig. 3A). There were no effects of FG 7142 on % open entries, total entries, closed entries, stretched-attend postures, or head-dips, irrespective of maze-experience ($F[1,44]$, all P's>0.07) (Fig. 3C–F). There was a significant main effect of maze-experience on head-dips ($F[1,44]=31.02$, $P<0.001$), with significantly lower head-dips in vehicle-treated maze-experienced than maze-naive counterparts (Fig. 3F).

3.4. Experiment 4: effects of flumazenil

ANOVA found no significant interaction between maze-experience and the effects of flumazenil ($F[3,77]$, all P's>0.23). There were no significant main effects of flumazenil ($F[3,77]$, all P's>0.34) for any measure (Fig. 4A–F). Although there was again a significant main effect of maze-experience on head-dips ($F[1,37]=10.42$, $P<0.001$), post-

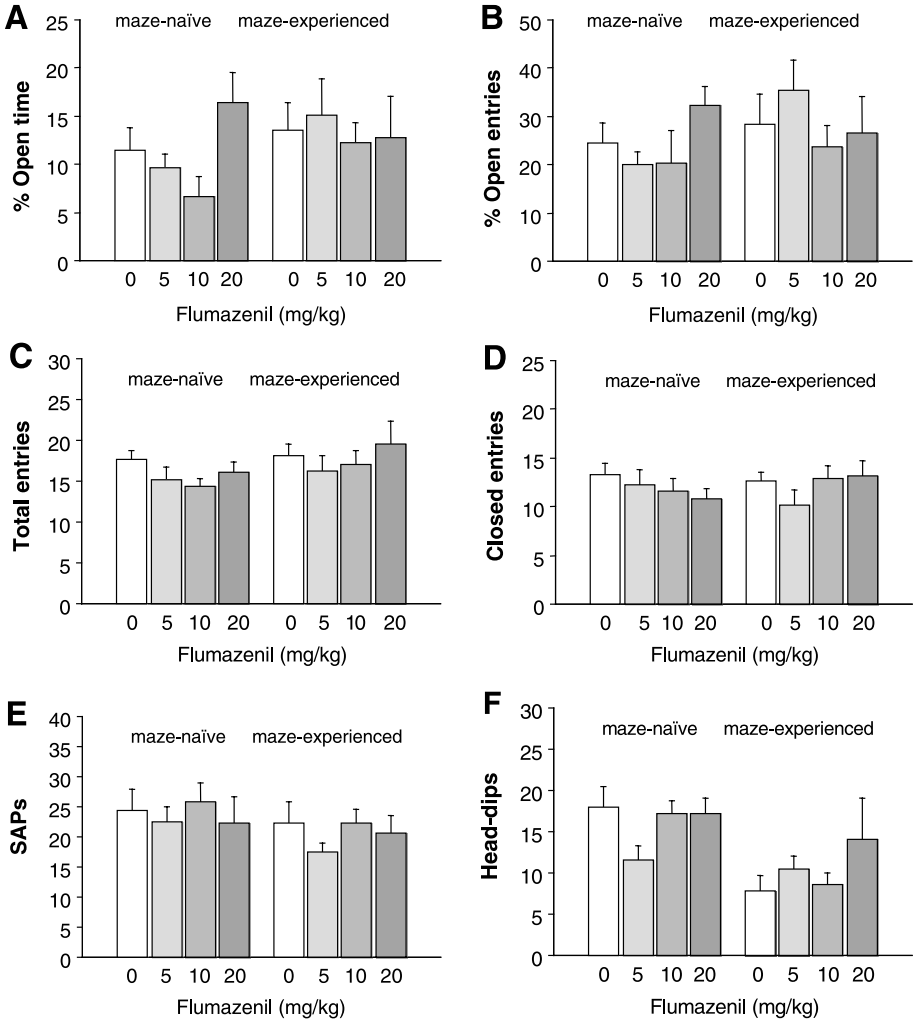

Fig. 4. The benzodiazepine antagonist, flumazenil, produced no effects on plus-maze behavior in either maze-naive or maze-experienced mice. Flumazenil produced no significant effect on plus-maze behavior, regardless of maze-experience (A–F) ($n=10–11$).

hoc comparison of vehicle control scores just failed to reach an acceptable level of statistical significance (Fig. 4F).

4. Discussion

The present study investigated the effects of plus-maze experience on subsequent behavioral responses to two monoaminergic anxiolytics. Results showed that plus-maze-experience significantly altered the plus-maze profiles of the monoamine oxidase inhibitor phenelzine and the serotonin reuptake inhibitor fluoxetine. A second aspect of the present study was to examine whether alterations in $GABA_A$/benzodiazepine receptor function may contribute to the altered behavioral profiles of phenelzine and fluoxetine occurring with maze-experience. The results of these experiments demonstrated that maze-experience produces a marked alteration in the anxiety-like effects of the benzodiazepine receptor partial inverse agonist, FG 7142, but did not alter the profile of the benzodiazepine receptor antagonist, flumazenil.

While there are some reports that phenelzine modifies anxiety-related behavior in various assays (e.g., Paslawski et al., 1996; Maki et al., 2000; Rupniak et al., 2000), the present finding is consistent with previous studies that have found no clear behavioral effects of acutely administered phenelzine on anxiety-like behavior in rodents (e.g., Johnston and File, 1998; Griebel et al., 1998; Beaufour et al., 1999). Thus, in mice that were naive to the plus-maze, low doses of phenelzine produced only trends for reduced open arm exploration. In contrast to the lack of clear effects in maze-naive mice, phenelzine produced a significant, dose-dependent increase in anxiety-like behavior in mice that had received a single prior undrugged exposure to the maze 24 h earlier. Compared to vehicle control, phenelzine significantly reduced the amount of time spent exploring the open arms of the plus-maze in maze-experienced mice. Phenelzine's effects on open arm avoidance were not associated with alterations in general locomotor activity, as demonstrated by the absence of a drug effect on closed arm entries. Furthermore, measures of exploratory head-dipping and risk assessment (stretched-attend postures) were not affected by phenelzine in maze-experienced (or maze-naive) mice.

Effects of maze-experience on baseline anxiety-like behavior have been observed in some but not all previous studies (for reviews, see Holmes and Rodgers, 1999; File, 2001). In our phenelzine study, there were very few behavioral differences between maze-naive and maze-experienced vehicle-control groups. Indeed throughout the present experiments effects of maze-experience on baseline behavior were largely absent. One interesting exception was a consistent decrease in head-dipping seen in maze-experienced mice relative to maze-naive controls, which may reflect an anxiety-related reduction in exploratory behavior on retesting, as previously reported (e.g., Fernandes and File, 1996; Rodgers et al., 1997; Holmes and Rodgers, 1998). Notwithstanding this one behavioral change, differential effects of phenelzine (and other compounds tested in the present study) seen between maze-experienced versus maze-naive mice are unlikely to be an artifact of divergent baseline scores occurring with maze-experience.

The effects of acutely administered fluoxetine in anxiety tests, including the elevated plus-maze, have proven inconsistent with reports of anxiogenic, anxiolytic and negative effects with this compound (for review, see Griebel, 1995). In the present study we found that acute fluoxetine had no clear effects on plus-maze behavior in maze-naive mice. Fluoxetine, particularly at the highest (20 mg/kg) dose, did produce reductions in all behavioral measures in maze-naive subjects that without exception failed to reach statistical significance. In parallel with the influence of maze-experience on the effects of phenelzine, prior maze-experience clearly strengthened the effects of fluoxetine. In contrast to the weak effects of fluoxetine in maze-naive mice, fluoxetine produced statistically robust effects on plus-maze behavior in mice with prior test experience. In maze-experienced subjects, fluoxetine induced a dose-dependent decrease in open arm exploration, as well as a decrease in head-dipping. In addition to changes in indices of anxiety-like behavior, fluoxetine also reduced closed arm entries and stretch-attend postures in maze-experienced mice. Reductions in open arm exploration coupled with fewer entries into the protected closed arms suggests a non-specific decrease in locomotor activity in maze-experienced mice treated with fluoxetine. An alternate explanation is that fluoxetine produced a strong anxiety-like response in maze-experienced mice that manifest as an overall suppression of plus-maze behavior. In support of this interpretation is the finding that high levels of fear and anxiety-like behavior can manifest as freezing and a general suppression of exploratory behavior (Blanchard and Blanchard, 1969; Blanchard et al., 1993), and the previous observation that sedative/motoric effects of fluoxetine in mice are generally seen at higher doses than presently used (e.g., Bourin et al., 1996; Belzung et al., 2001).

Notwithstanding this issue, the main conclusion of these experiments is that prior experience of the plus-maze leads to an increased sensitivity to the acute behavioral effects of both phenelzine and fluoxetine.

Previous research has suggested that functional changes in monoamine receptors may underlie experientially induced alterations in drug response in the elevated plus-maze test. File et al. have found that, in maze-experienced (but not maze-naive) rats, microinjection of the $5\text{-}HT_{1A}$ receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) into the dorsal raphé nucleus produces anxiolytic effects (File and Gonzalez, 1996), whereas microinjection into the dorsal hippocampus has the opposite (i.e., anxiogenic-like) effect (File et al., 1996). These authors suggest that prior exposure to the maze results in an increased functional sensitivity of $5\text{-}HT_{1A}$ receptors at both presynaptic (resulting in enhanced negative feedback inhibition of serotonergic neuronal firing) and postsynaptic sites (resulting in

enhanced inter-neuronal serotonergic signaling). In the context of present findings, acute administration of both phenelzine and fluoxetine would produce a significant increase in extracellular levels of serotonin (e.g., Blier and de Montigny, 1985; Dyck et al., 1988; Kreiss and Lucki, 1995; but see Artigas, 2001; Artigas et al., 2001) and a corresponding activation of presynaptic 5-HT_{1A} autoreceptors (Sharp and Hjorth 1990; Blier et al. 1998). Hypersensitization of 5-HT_{1A} autoreceptors with maze-experience would augment these actions, causing an enhancement of 5-HT_{1A} autoreceptor-mediated inhibition of serotonergic neuronal firing. This does not rule out the possibility that maze-experience alters the activity of these drugs at other sites in the brain (e.g., 5-HT_{2C} receptors) which could also contribute to their altered behavioral profiles in maze-experienced mice.

Anxiogenic effects of acute phenelzine and fluoxetine following maze-experience is consistent with the initial pro-anxiety effects of monoaminergic anxiolytics in some patients with mood disorders (e.g., Rickels et al., 1985; Londborg et al., 2000). Thus, relative to the behavior of maze-naive subjects, the form of anxiety-like behavior measured in maze-experienced mice may better model the short-term adverse effects of monoaminergic anxiolytics. In view of the evidence that treatment with a 5-HT_{1A} receptor antagonist can alleviate the short-term side effects of monoaminergic anxiolytics (e.g., Artigas et al., 1996), it would valuable to test whether anxiogenic effects of phenelzine and fluoxetine in maze-experienced mice can be blocked by compounds such as pindolol. Supporting the possibility that the behavioral effects of 5-HT_{1A} receptor antagonists may be altered by maze-experience, recent research from our laboratory has shown that microinjection of the 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide dihydrochloride (WAY-100635) into the ventral hippocampus or median raphé nucleus produces anxiolytic effects in maze-naive mice that are abolished by maze-experience (Canto de Souza et al., 2002; Nunes de Souza et al., 2002).

Another system that could contribute to the alterations in the anxiety-related effects of phenelzine and fluoxetine seen with maze-experience is the GABA/benzodiazepine receptor complex. Facilitation of GABAergic neurotransmission has been proposed as a possible mechanism underlying the anxiolytic effects of both monoamine oxidase inhibitors (e.g., Paslawski et al., 1996; Tanay et al., 2001; Lai et al., 1998; Parent et al., 2002) and serotonin reuptake inhibitors (e.g., Uzunova et al., 1998; Tunnicliff et al., 1999; Belzung et al., 2001; Matsubara et al., 2000). Moreover, a widely replicated finding in both rats and mice has been that the anxiolytic effects of benzodiazepine agonists, such as diazepam, midazolam and chlordiazepoxide, are abolished by maze-experience (for reviews, see Holmes and Rodgers, 1999; File, 2001), and plus-maze experience in rats has been shown to alter the cortical release of GABA following benzodiazepine treatment (File, 1993). Together these findings suggest that a fundamental change in $GABA_A$/benzodiazepine receptor function may contribute to the altered behavioral effects of monoaminergic anxiolytics with retesting.

Some support for this hypothesis is provided by our finding that the selective anxiogenic effects of a benzodiazepine receptor partial inverse agonist, FG 7142 (e.g., Lister, 1987; File and Zangrossi, 1993; Cole et al., 1995; Dawson et al., 1995; Rodgers et al., 1995), were abolished in maze-experienced mice. However, the hypothesis is not supported by the separate finding that the benzodiazepine receptor antagonist, flumazenil, was inactive on the plus-maze in both maze-naive and maze-experienced mice. Thus, a shift in the functional status of the benzodiazepine receptor occurring with retesting would be expected to result in an observable change in the anxiety-related profile of flumazenil in maze-experienced mice (Nutt et al., 1992; Nutt and Malizia, 2001). An alternative explanation for this finding, which could also underlie both the loss of benzodiazepine agonist-induced anxiolysis and benzodiazepine inverse agonist-induced anxiogensis occurring with plus-maze retesting, is that GABA/benzodiazepine mechanisms are not recruited during plus-maze exposure in subjects that have been previously tested on the apparatus.

In summary, present findings show that a single undrugged experience of the elevated plus-maze modifies and strengthens the behavioral actions of two monoaminergic anxiolytics. Acute treatment with the monoamine oxidase inhibitor phenelzine produced selective anxiogenic effects in maze-experienced mice, whereas the drug had no clear effects in maze-naive subjects. Acute administration of the serotonin reuptake inhibitor fluoxetine also produced dose-dependent effects on indices of anxiety and motor behavior in maze-experienced mice, but had only partial effects in maze-naive subjects. It will be of considerable interest to test whether maze-experience also sensitizes the plus-maze effects of these compounds when given chronically. One hypothetical mechanism underlying the heightened sensitivity to phenelzine and fluoxetine following maze-experience is an increased functional sensitivity of the 5-HT_{1A} receptor. Clear alterations in $GABA_A$/benzodiazepine receptor function do not appear to contribute to the effects of maze-experience. In the present study we demonstrated that the anxiogenic actions of the benzodiazepine receptor inverse agonist FG 7142 were lost in maze-experienced mice, however, the benzodiazepine receptor antagonist, flumazenil, was silent in both maze-naive and maze-experienced mice.

A number of important general considerations all stem from the present study. Clearly, care should be exercised when testing mice on the elevated plus-maze to ensure that prior test history is carefully controlled. In pharmacological studies, inconsistency in the use of maze-naive versus maze-experienced subjects may contribute to inter-laboratory variability in the anxiety-related effects of monoaminergic drugs (Griebel, 1995; Hogg, 1996; Rodgers, 1997). This issue is of particular relevance for research using gene mutant mice to study molecular mechanisms mediating anxiety, where the

need to economize on the use of subjects may lead to multiple test exposures. Apart from the practical caveats of repeated plus-maze testing, the effects of maze-experience may provide important insights into the pathophysiology of anxiety states. Behavior in maze-experienced mice may reflect a qualitatively distinct form of anxiety from that seen in maze-naive mice (Holmes and Rodgers, 1998; File, 2001). The increased sensitivity to acute monoaminergic anxiolytics and the loss of sensitivity to benzodiazepine receptor anxiolytics that occurs with maze-experience parallel the psychopharmacology of certain anxiety disorders. From this perspective, the behavior of plus-maze behavior in maze-experienced mice may serve as an independent model to study the brain mechanisms underlying different anxiety states and their treatment.

Acknowledgements

A.H. was supported by a School of Psychology (University of Leeds) research studentship.

References

Artigas, F., 2001. Limitations to enhancing the speed of onset of antidepressants—are rapid action antidepressants possible? Hum. Psychopharmacol. Clin. Exp. 16, 29–36.

Artigas, F., Romero, L., de Montigny, C., Blier, P., 1996. Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT_{1A} antagonists. Trends Neurosci. 19, 378–383.

Artigas, F., Celada, P., Laruelle, M., Adell, A., 2001. How does pindolol improve antidepressant action? Trends Pharmacol. Sci. 22, 224–228.

Ballenger, J.C., 1999. Current treatments of the anxiety disorders in adults. Biol. Psychiatry 46, 1579–1594.

Beaufour, C.C., Ballon, N., Le Bihan, C., Hamon, M., Thiebot, M.H., 1999. Effects of chronic antidepressants in an operant conflict procedure of anxiety in the rat. Pharmacol. Biochem. Behav. 62, 591–599.

Belzung, C., Le Guisquet, A.M., Barreau, S., Calatayud, F., 2001. An investigation of the mechanisms responsible for acute fluoxetine-induced anxiogenic-like effects in mice. Behav. Pharmacol. 12, 151–162.

Blakely, R.D., Berson, H.E., Fremeau Jr., R.T., Caron, M.G., Peek, M.M., Prince, H.K., Bradley, C.C., 1991. Cloning and expression of a functional serotonin transporter from rat brain. Nature 354, 66–70.

Blanchard, R.J., Blanchard, D.C., 1969. Crouching as an index of fear. J. Comp. Physiol. Psychol. 67, 370–375.

Blanchard, R.J., Yudko, E.B., Rodgers, R.J., Blanchard, D.C., 1993. Defense system psychopharmacology: an ethological approach to the pharmacology of fear and anxiety. Behav. Brain Res. 58, 155–165.

Blier, P., de Montigny, C., 1985. Serotoninergic but not noradrenergic neurons in rat central nervous system adapt to long-term treatment with monoamine oxidase inhibitors. Neuroscience 16, 949–955.

Blier, P., Pineyro, G., el Mansari, M., Bergeron, R., de Montigny, C., 1998. Role of somatodendritic 5-HT autoreceptors in modulating 5-HT neurotransmission. Ann. N. Y. Acad. Sci. 861, 204–216.

Bourin, M., Redrobe, J.P., Hascoet, M., Baker, G.B., Colombel, M.C., 1996. A schematic representation of the psychopharmacological profile of antidepressants. Prog. Neuropsychopharmacol. Biol. Psychiatry 20, 1389–1402.

Canto de Souza, A., Nunes de Souza, R.L., Rodgers, R.J., 2002. Anxiolytic-like effect of WAY-100635 microinfusions into the median (but not dorsal) raphe nucleus in mice exposed to the plus-maze: influence of prior test experience. Brain Res. 928, 50–59.

Cole, B.J., Hillmann, M., Seidelmann, D., Klewer, M., Jones, G.H., 1995. Effects of benzodiazepine receptor partial inverse agonists in the elevated plus maze test of anxiety in the rat. Psychopharmacology 121, 118–126.

Crawley, J.N., Paylor, R., 1997. A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. Horm. Behav. 31, 197–211.

Dalvi, A., Rodgers, R.J., 1999. Behavioral effects of diazepam in the murine plus-maze: flumazenil antagonism of enhanced head dipping but not the disinhibition of open-arm avoidance. Pharmacol. Biochem. Behav. 62, 727–734.

Dawson, G.R., Crawford, S.P., Collinson, N., Iversen, S.D., Tricklebank, M.D., 1995. Evidence that the anxiolytic-like effects of chlordiazepoxide on the elevated plus maze are confounded by increases in locomotor activity. Psychopharmacology 118, 316–323.

Dyck, L.E., Juorio, A.V., Durden, D.A., Boulton, A.A., 1988. Effect of chronic deuterated and non-deuterated phenelzine on rat brain monoamines and monoamine oxidase. Naunyn-Schmiedeberg's Arch. Pharmacol. 337, 279–283.

Fernandes, C., File, S.E., 1996. The influence of open arm ledges and maze experience in the elevated plus-maze. Pharmacol. Biochem. Behav. 54, 31–40.

File, S.E., 1990. One-trial tolerance to the anxiolytic effects of chlordiazepoxide in the plus-maze. Psychopharmacology 100, 281–282.

File, S.E., 1993. The interplay of learning and anxiety in the elevated plus-maze. Behav. Brain Res. 58, 199–202.

File, S.E., 2001. Factors controlling measures of anxiety and responses to novelty in the mouse. Behav. Brain Res. 125, 151–157.

File, S.E., Gonzalez, L.E., 1996. Anxiolytic effects in the plus-maze of 5-HT_{1A}-receptor ligands in dorsal raphe and ventral hippocampus. Pharmacol. Biochem. Behav. 54, 123–128.

File, S.E., Zangrossi, H., 1993. 'One-trial tolerance' to the anxiolytic actions of the benzodiazepines in the elevated plus-maze, or the development of a phobic state? Psychopharmacology 110, 240–244.

File, S.E., Mabbutt, P.S., Hitchcott, P.K., 1990. Characterisation of the phenomenon of one-trial tolerance to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze. Psychopharmacology 102, 98–101.

File, S.E., Zangrossi Jr., H., Sanders, F.L., Mabbutt, P.S., 1994. Raised corticosterone in the rat after exposure to the elevated plus-maze. Psychopharmacology 113, 543–546.

File, S.E., Gonzalez, L.E., Andrews, N., 1996. Comparative study of pre- and postsynaptic 5-HT_{1A} receptor modulation of anxiety in two ethological animal tests. J. Neurosci. 16, 4810–4815.

File, S.E., Gonzalez, L.E., Gallant, R., 1998. Role of the basolateral nucleus of the amygdala in the formation of a phobia. Neuropsychopharmacology 19, 397–405.

Griebel, G., 1995. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol. Ther. 65, 319–395.

Griebel, G., Rodgers, R.J., Perrault, G., Sanger, D.J., 1997. Risk assessment behaviour: evaluation of utility in the study of 5-HT-related drugs in the rat elevated plus-maze. Pharmacol. Biochem. Behav. 57, 817–827.

Griebel, G., Curet, O., Perrault, G., Sanger, D.J., 1998. Behavioral effects of phenelzine in an experimental model for screening anxiolytic and anti-panic drugs: correlation with changes in monoamine-oxidase activity and monoamine levels. Neuropharmacology 37, 927–935.

Handley, S.L., Mithani, S., 1984. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. Naunyn-Schmiedeberg's Arch. Pharmacol. 327, 1–5.

Hascoet, M., Bourin, M., Couetoux du Tertre, A.C., 1997. Influence of prior experience on mice behavior using the four-plate test. Pharmacol. Biochem. Behav. 58, 1131–1138.

Hogg, S., 1996. A review of the validity and variability of the elevated plus-

maze as an animal model of anxiety. Pharmacol. Biochem. Behav. 54, 21–30.
Holmes, A., 2001. Transgenic and gene knockout approaches to the study of anxiety-like behavior in mice. Neurosci. Biobehav. Rev. 25, 261–273.
Holmes, A., Rodgers, R.J., 1998. Responses of Swiss-Webster mice to repeated plus-maze experience: further evidence for a qualitative shift in emotional state? Pharmacol. Biochem. Behav. 60, 473–488.
Holmes, A., Rodgers, R.J., 1999. Influence of spatial and temporal manipulations on the anxiolytic efficacy of chlordiazepoxide in mice previously exposed to the elevated plus-maze. Neurosci. Biobehav. Rev. 23, 971–980.
Holmes, A., Rodgers, R.J., 2001. Prior test experience abolishes the anxiolytic efficacy of chlordiazepoxide in the mouse light/dark exploration test. Behav. Brain Res. 122, 159–167.
Holmes, A., Diffley, E.P., Walton, T.J., Brain, P.F., Rodgers, R.J., 1998. Lack of habituation of corticosterone response in mice repeatedly exposed to the plus-maze. J. Psychopharmacol. 12S, A32.
Holmes, A., Parmigiani, S., Ferrari, P.F., Palanza, P., Rodgers, R.J., 2000. Behavioral profile of wild mice in the elevated plus-maze test for anxiety. Physiol. Behav. 71, 509–516.
Johnston, A.L., File, S.E., 1998. Profiles of the antipanic compounds, triazolobenzodiazepines and phenelzine, in two animal tests of anxiety. Psychiatry Res. 25, 81–90.
Kreiss, D.S., Lucki, I., 1995. Effects of acute and repeated administration of antidepressant drugs on extracellular levels of 5-hydroxytryptamine measured in vivo. J. Pharmacol. Exp. Ther. 274, 866–876.
Lai, C.T., Tanay, V.A., Charrois, G.J., Baker, G.B., Bateson, A.N., 1998. Effects of phenelzine and imipramine on the steady-state levels of mRNAs that encode glutamic acid decarboxylase (GAD67 and GAD65), the GABA transporter GAT-1 and GABA transaminase in rat cortex. Naunyn-Schmiedeberg's Arch. Pharmacol. 357, 32–38.
Liebowitz, M.R., Hollander, E., Schneier, F., Campeas, R., Welkowitz, L., Hatterer, J., Fallon, B., 1990. Reversible and irreversible monoamine oxidase inhibitors in other psychiatric disorders. Acta Psychiatr. Scand. 360, 29–34.
Lister, R.G., 1987. The use of the plus-maze to measure anxiety in the mouse. Psychopharmacology 92, 180–185.
Londborg, P.D., Smith, W.T., Glaudin, V., Painter, J.R., 2000. Short-term cotherapy with clonazepam and fluoxetine: anxiety, sleep disturbance and core symptoms of depression. J. Affect. Disord. 61, 73–79.
Maki, Y., Inoue, T., Izumi, T., Muraki, I., Ito, K., Kitaichi, Y., Li, X., Koyama, T., 2000. Monoamine oxidase inhibitors reduce conditioned fear stress-induced freezing behavior in rats. Eur. J. Pharmacol. 406, 411–418.
Matsubara, M., Suzuki, S., Miura, K., Terashima, M., Sugita, S., Kimura, H., Hatsuda, S., Mori, T., Murakami, H., Hayashi, T., Ohta, T., Ohara, M., 2000. Electrophysiologic analysis of antidepressant drug effects on the GABA(A) receptor complex based upon antagonist-induced encephalographic power spectrum changes. Neuropsychobiology 42, 149–157.
McGregor, I.S., Dielenberg, R.A., 1999. Differential anxiolytic efficacy of a benzodiazepine on first versus second exposure to a predatory odor in rats. Psychopharmacology 147, 174–181.
Mcllwain, K.L., Merriweather, M.Y., Yuva-Paylor, L.A., Paylor, R., 2001. The use of behavioral test batteries: effects of training history. Physiol. Behav. 73, 705–717.
Nunes de Souza, R.L., Canto de Souza, A., Rodgers, R.J., 2002. Effects of intra-hippocampal infusion of WAY-100635 on plus-maze behavior in mice: influence of site of injection and prior test experience. Brain Res. 927, 87–96.
Nutt, D.J., Malizia, A.L., 2001. New insights into the role of the GABA(A)-benzodiazepine receptor in psychiatric disorder. Br. J. Psychiatry 179, 390–396.
Nutt, D.J., Smith, C.F., Bennett, R., Jackson, H.C., 1992. Investigations on the "set-point" theory of benzodiazepine receptor function. Adv. Biochem. Psychopharmacol. 47, 419–429.
Parent, M.B., Master, S., Kashlub, S., Baker, G.B., 2002. Effects of the antidepressant/antipanic drug phenelzine and its putative metabolite phenylethylidenehydrazine on extracellular gamma-aminobutyric acid levels in the striatum. Biochem. Pharmacol. 63, 57–64.
Paslawski, T., Treit, D., Baker, G.B., George, M., Coutts, R.T., 1996. The antidepressant drug phenelzine produces antianxiety effects in the plus-maze and increases in rat brain GABA. Psychopharmacology 127, 19–24.
Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open:-closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14, 149–167.
Rickels, K., Smith, W.T., Glaudin, V., Amsterdam, J.B., Weise, C., Settle, G.P., 1985. Comparison of two dosage regimens of fluoxetine in major depression. J. Clin. Psychiatry 46, 38–41.
Rodgers, R.J., 1997. Animal models of 'anxiety': where next? Behav. Pharmacol. 8, 477–496.
Rodgers, R.J., Cole, J.C., 1994. The elevated plus-maze: pharmacology, methodology and ethology. In: Cooper, S.J., Hendrie, C.A. (Eds.), Ethology and Psychopharmacology. Wiley, Chichester, pp. 9–44.
Rodgers, R.J., Shepherd, J.K., 1993. Influence of prior maze experience on behaviour and response to diazepam in the elevated plus-maze and the light/dark tests of anxiety in mice. Psychopharmacology 113, 237–242.
Rodgers, R.J., Lee, C., Shepherd, J.K., 1992. Effects of diazepam on behavioural and antinociceptive responses to the elevated plus-maze in male mice depend upon treatment regimen and prior maze experience. Psychopharmacology 106, 102–110.
Rodgers, R.J., Cole, J.C., Aboualfa, K., Stephenson, L.H., 1995. Ethopharmacological analysis of the effects of putative 'anxiogenic' agents in the mouse elevated plus-maze. Pharmacol. Biochem. Behav. 52, 805–813.
Rodgers, R.J., Johnson, N.J., Cole, J.C., Dewar, C.V., Kidd, G.R., Kimpson, P.H., 1996. Plus-maze retest profile in mice: importance of initial stages of trail 1 and response to post-trail cholinergic receptor blockade. Pharmacol. Biochem. Behav. 54, 41–50.
Rodgers, R.J., Johnson, N.J.T., Carr, J., Hodgson, T.P., 1997. Resistance of experientially induced changes in murine plus-maze behaviour to altered retest conditions. Behav. Brain Res. 86, 1–77.
Rodgers, R.J., Haller, J., Holmes, A., Halasz, J., Walton, T., Brain, P.F., 1999. Corticosterone response to the plus-maze: high correlation with risk assessment in rats and mice. Physiol. Behav. 68, 47–53.
Rogers, D.C., Fisher, E.M., Brown, S.D., Peters, J., Hunter, A.J., Martin, J.E., 1997. Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. Mamm. Genome 8, 711–713.
Rupniak, N.M., Carlson, E.C., Harrison, T., Oates, B., Seward, E., Owen, S., de Felipe, C., Hunt, S., Wheeldon, A., 2000. Pharmacological blockade or genetic deletion of substance P (NK(1)) receptors attenuates neonatal vocalisation in guinea-pigs and mice. Neuropharmacology 39, 1413–1421.
Sharp, T., Hjorth, S., 1990. Application of brain microdialysis to study the pharmacology of the 5-HT_{1A} autoreceptor. J. Neurosci. Methods 34, 83–90.
Stein, D.J., Berk, M., 2000. Selective serotonin reuptake inhibitors in the anxiety disorders. Int. Clin. Psychopharmacol. 15, S1–S63.
Stokes, P.E., Holtz, A., 1997. Fluoxetine tenth anniversary update: the progress continues. Clin. Ther. 19, 1135–1250.
Tanay, V.M., Greenshaw, A.J., Baker, G.B., Bateson, A.N., 2001. Common effects of chronically administered antipanic drugs on brainstem GABA(A) receptor subunit gene expression. Mol. Psychiatry 6, 404–412.
Tunnicliff, G., Schindler, N.L., Crites, G.J., Goldenberg, R., Yochum, A., Malatynska, E., 1999. The GABA(A) receptor complex as a target for fluoxetine action. Neurochem. Res. 24, 1271–1276.
Uzunova, V., Sheline, Y., Davis, J.M., Rasmusson, A., Uzunov, D.P., Costa, E., Guidotti, A., 1998. Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. Proc. Natl. Acad. Sci. U. S. A. 95, 3239–3244.