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Strain- and model-dependent effects of chlordiazepoxide, L-838,417 and zolpidem on anxiety-like behaviours in laboratory mice

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Abstract

The promise of subtype-selective $GABA_A$ receptor drugs with anxiolytic properties but with a much reduced side-effect burden (compared to benzodiazepines) is an attainable goal. However, its achievement necessitates the availability of in vivo preclinical assays capable of demonstrating differences as well as similarities between subtype-selective agents and non-selective benzodiazepines. In this study, we have compared three mouse strains (NMRI, C57BL/6J and DBA/2) in four models of anxiety-like behaviour (plus-maze, zero-maze, light–dark, and Vogel conflict). Furthermore, in each model, we have contrasted in detail the behavioural responses of each strain to the non-selective benzodiazepine chlordiazepoxide (CDP; 5–20 mg/kg), and the subtype-selective agents L-838,417 ($GABA_A$-$\alpha_{2/3/5}$; 3–30 mg/kg) and zolpidem ($GABA_A$-α1; 0.3–3.0 mg/kg). The data show a complex mouse strain × model × pharmacological agent interaction. Most importantly, not all mouse strain × model test systems showed a positive response to CDP or predicted the response to L-838,417. This dissociation between CDP and L-838,417 opens up opportunities for preclinical test systems that differentiate subtype-selective and non-selective $GABA_A$ receptor agents, an attribute that might well be important in providing the necessary confidence for further drug development. Present findings suggest the need for a much greater focus on defining test systems appropriate for screening novel chemical entities, rather than self-selection of models or genotypes based on responses to known pharmacological agents. For example, if current data with L-838,417 are confirmed with compounds showing similar selectivity profiles, such agents may in future be best identified and characterised using test systems comprising NMRI mice in the zero-maze and/or C57 mice in the Vogel conflict and/or light–dark tests.

Keywords: Models of anxiety-like behaviour; $GABA_A$ receptor; α-subunits; Chlordiazepoxide; L-838,417; Zolpidem; Genotype; Mouse

1. Introduction

Although the functional relationship between benzodiazepine (BZ) binding sites and $GABA_A$ receptors has been known for some 30 years, the past decade has witnessed major advances in our understanding of the molecular mechanisms underlying the behavioural pharmacology of BZs. It is now known that the $GABA_A$ receptor comprises 5 protein subunits (typically 2α, 2β and 1γ) surrounding a central chloride ionophore (Barnard et al., 1998), with strong evidence to suggest that the precise protein composition of this receptor is of crucial importance to the action of BZs and related drugs (Mohler et al., 2002; Sieghart, 2006). One of the principal sources of evidence derives from techniques in molecular biology that have enabled the creation of genetically-modified mice (Crawley, 2000). Although gene knockout has been widely used to study the functional significance of particular gene products (e.g. Nelson and Young, 1998; Weiss et al., 2000; Holmes, 2001; Cryan and Holmes, 2005), gene 'knock-in' has been more widely adopted in research into $GABA_A$ receptors and the mode of action of BZs (Rudolph et al., 1999; Löw et al., 2000; McKernan et al., 2000; Crestani et al., 2001).

Of particular relevance to anxiety research are studies involving mice with knock-in point mutations in genes for the

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various α-subunits of the $GABA_A$ receptor. Changing the amino acid histidine to arginine at position 101 of the α_1 gene renders this subunit insensitive to BZ binding without altering GABA sensitivity or receptor expression (Wieland et al., 1992; Kleingoor et al., 1993; Benson et al., 1998). Intriguingly, however, α_1 knock-in mice are insensitive to the sedative, amnestic and (to some extent) anti-convulsant effects of diazepam (DZ), but retain normal muscle-relaxant, ethanol-potentiating and anxiolytic responses to the compound (Rudolph et al., 1999). These findings (see also Crestani et al., 2000; McKernan et al., 2000) clearly indicate a major role for $GABA_A$-α_1 receptors in the sedative, but not anxiolytic, effects of DZ. Subsequent research using α_2 and α_3 knock-in mice has shown that these animals retain normal locomotor suppressant, ataxic and anti-convulsant responses to DZ, indirectly confirming the importance of α_1 subunits to these particular effects of DZ (Löw et al., 2000). Significantly, however, while α_3 mutant mice retained sensitivity to the anxiolytic effects of DZ, α_2 mutant mice failed to display this response. Thus, complementing the α_1 knock-in phenotype, these data indicate a major role for $GABA_A$-α_2 receptors in the anxiolytic, but not sedative, effects of DZ.

This new understanding of $GABA_A$ receptor mechanisms has naturally stimulated efforts to develop novel anxiolytic compounds via a) selectivity to $GABA_A$ receptor α-subunits associated with anxiety relative to those mediating sedation, and/or b) increased efficacy at α_2 and/or α_3 subtypes (Atack, 2003). For example, L-838,417 acts as a partial $\alpha_{2/3/5}$ selective agonist and an α_1 antagonist and, consistent with the genetic research above, has behaviourally-selective anxiolytic-like effects in the rat elevated plus-maze (1–10 mg/kg) and fear-potentiated startle (0.3–3 mg/kg) tests (McKernan et al., 2000). More recently, other subtype-selective $GABA_A$ positive modulators have been developed which also reduce anxiety-like behaviour in rodents at doses that do not produce unwanted side-effects in tests of sedation and muscle strength. These compounds include SL651498, a full agonist at $GABA_A$-$\alpha_{2/3}$ receptors and a partial $\alpha_{1/5}$ agonist (Griebel et al., 2001); TPA023, a partial agonist at $GABA_A$-$\alpha_{2/3}$ receptors ($\alpha_3 > \alpha_2$) and an antagonist at $\alpha_{1/5}$ receptors (Atack et al., 2006); and TP003 which has selective efficacy at $GABA_A$-α_3 receptors (Dias et al., 2005). Although ocinaplon, a low affinity BZ site ligand with modest selectivity for $GABA_A$-α_1 receptors, exerts an "anxioselective" profile in man (Chilman-Blair et al., 2003), Phase III clinical trials for generalised anxiety disorder have been halted due to potential hepatotoxicity (Basile et al., 2004; Lippa et al., 2005).

Prior to the present series of experiments, the anxiolytic-like effects of L-838,417 had only been demonstrated in the rat plus-maze and fear-potentiated startle tests. Since the in vivo functions of $GABA_A$ receptor α_1, α_2 and α_3 subtypes had been established in mice (above), it seemed essential to characterise this novel compound in murine tests of anxiety-like behaviour. More specifically, our aim was to contrast the behavioural effects in mice of this $GABA_A$-α subtype-selective ligand with those of a non-selective classical BZ (chlordiazepoxide; CDP) and a $GABA_A$-α_1-selective compound (zolpidem; ZOL). In view of major differences in basal anxiety and BZ sensitivity in commonly used mouse strains (e.g. Trullas and Skolnick, 1993; Mathis et al., 1994; Crawley et al., 1997; Homanics et al., 1999; Rogers et al., 1999; Crabbe et al., 1999; Crawley, 2000; Griebel et al., 2000; Tarantino et al., 2000; Bolivar et al., 2000; Belzung, 2001; van Gaalen and Steckler, 2000; Contet et al., 2001; Rodgers et al., 2002; Voikar et al., 2004), the dose-response effects of these three compounds were determined in three mouse strains selected on the basis of contrasting behavioural phenotypes ('low emotion' inbred C57BL/6J versus 'high emotion' inbred DBA/2) and/or widespread use in behavioural research (outbred NMRI Swiss). Finally, given the evidence for substantial variation in the behavioural pharmacology of different models of anxiety-like behaviour (e.g. Rodgers, 1997), four tests were chosen to overlap with and to extend the range of procedures commonly used in this field of research i.e. the elevated plus-maze (EPM; Lister, 1987), light/dark exploration (LDE; Crawley and Goodwin, 1980), elevated zero-maze (ZM; Shepherd et al., 1994), and Vogel conflict (VCT; Vogel et al., 1971) tests.

2. Materials and methods

2.1. Ethics

Studies conducted at Leeds University (EPM and LDE) were licenced by the Home Office under the Animals (Scientific Procedures) Act 1986, while those conducted at NeuroSearch A/S (ZM and VCT) were licenced by the Danish Committee for Experiments on Animals under the Act of the Prevention of Cruelty to Animals (2000). In view of the two-site nature of this study, every effort was made to standardise as many methodological variables as was practicable.

2.2. Subjects

Male outbred HsdWin:NMRI (National Marine Research Institute; NMRI), inbred C57BL/6JOlaHsD (C57) and inbred DBA/2OlaHsd (DBA) mice were supplied by Harlan Netherlands or M&B Breeding and Research Centre Denmark (for studies at NeuroSearch A/S) or by Harlan UK (for studies at Leeds). On arrival (5–6 weeks old), mice were housed in same-strain groups of 7–10, and maintained under a normal 12-hour light cycle (lights on: 0600h or 0700h) in a temperature- (21±1° C) and humidity- (50±5%) controlled environment. At NeuroSearch A/S, cages were kept in closed ventilated animal cupboards (Scantainer; Scanbur A/S, Ejby, Denmark) that were moved to the test room one day prior to testing. At Leeds, cages were kept on open shelves in a holding room and transported to the test room at least 1 h prior to testing. Cage substrate (wood shavings) was changed twice weekly, with food and tap water freely available except where otherwise stated (see VCT methods). Animals were allowed to acclimatise for either 1–2 weeks (NeuroSearch A/S) or 4 weeks (Leeds) prior to use. Naïve animals were used in all experiments.

2.3. Drugs

Chlordiazepoxide (CDP) was obtained from Sigma-Aldrich (Vallensbæk Strand, Denmark), and zolpidem (ZOL) from

Tocris (Ellisville, Missouri, USA). L-838,417 was synthesised by the Department of Medicinal Chemistry at NeuroSearch A/S. All compounds were freshly prepared on experimental days by 30 min ultrasonic dispersion in a vehicle comprising 5% w/v of the emulsifying agent cremophor (Basis Kemi, Denmark) in deionised water. Dose ranges were selected on the basis of the published literature (e.g. Homanics et al., 1999; Griebel et al., 2000; McKernan et al., 2000; Olivier et al., 2002; Rodgers et al., 2002), and all drugs were administered intraperitoneally (i.p.) in a volume of 10 ml/kg 30 min prior to testing.

2.4. Apparatus and procedures

2.4.1. Elevated plus-maze (EPM)

The EPM apparatus was based on the design of Lister (1987) and comprised two open arms (30×5×0.25 cm) and two closed arms (30×5×15 cm) extending from a 5×5 cm central platform. The maze was constructed from Plexiglas (black floor, clear walls) and elevated 60 cm above floor level. The light level was 70 lx at maze level. The experiments were counterbalanced both for genetic strain and dose. After injection (n=10), mice were individually placed in holding cages (17×7×6 cm), which were also used for transport to the maze. Thirty minutes following injection, mice were individually placed on the central platform of the maze facing an open arm. The experimenter withdrew to an adjacent room during the 5 min test sessions, which were videorecorded by an overhead camera (angled at approximately 50° to the maze) linked to a VCR and monitor in an adjacent room. The maze was cleaned with damp and dry cloths between subjects. Test videotapes were subsequently scored blind using the ethological software Hindsight (Weiss, 1995). Behavioural parameters comprised conventional as well as ethological measures (see Rodgers et al., 2002). Conventional measures were the frequencies of total, open and closed entries (arm entry=all four paws into an arm), % open entries [(open/total)×100), and % time spent in open, closed and central parts of the maze [e.g. (time open/session duration)×100]. Ethological measures comprised frequency scores for supported rearing (vertical movement against the side/end walls; mice very rarely exhibit unsupported rearing in this test), head-dipping (exploratory movement of head/shoulders over the side of the maze), and stretched-attend postures (SAP: exploratory posture in which the body is stretched forward then retracted to the original position without any forward locomotion). In view of the importance of thigmotactic cues to rodent exploration, head-dipping and SAP were also differentiated as a function of their occurrence in different parts of the maze. Thus, the closed arms and centre platform were designated as "protected" areas (i.e. offering relative security) and the "percent protected" scores for head-dipping and SAP were calculated as the percentage of these behaviours displayed in or from the protected areas (e.g. [(protected SAP/total SAP)×100].

2.4.2. Elevated zero-maze (ZM)

The ZM apparatus (TSE Systems, Germany), based on that described for rats by Shepherd et al. (1994), was a ring-shaped runway (inner diameter=47 cm) elevated 50 cm above floor level. The runway had a black Plexiglas floor (2.8 cm width) and comprised alternating open and closed quadrants. The closed quadrants were surrounded by an 11.1 cm high clear Plexiglas wall, whereas the open quadrants were flanked by a 6 mm Plexiglas lip. Black plastic strips were glued on the outside of these lips to facilitate their visibility (this has previously been shown to encourage open area exploration; B. Hartz, pers. comm.). Testing took place under dim white light (5 lx at maze level). Test order was counterbalanced for both genetic strain and drug treatment. Mice (n=5–12) were tail-marked, injected with vehicle or test compound and returned to the home cage. Thirty minutes later, they were individually placed on the maze facing a closed quadrant and allowed 5 min exploration of the apparatus. The maze was cleaned with a damp and a dry tissue after every 10th mouse and, between experiments, with 70% ethanol. The observer was situated ~50 cm from the maze, with the position of the mouse and its behaviour scored on a PC using ethological software (Noldus Observer program, version 2.01; Noldus Information Technology, Wageningen, The Netherlands). Measures recorded were: latency to enter an open quadrant; number of entries into open quadrants; time spent in open quadrants; number of stretched-attend postures in closed quadrants (protected SAP); number of head-dips in open quadrants; and frequency of rearings (for full definitions, see Korsgaard et al., 2004;Troelsen et al., 2005).

2.4.3. Light–dark exploration (LDE)

This apparatus was based on that reported by Crawley and Goodwin (1980). An open-top arena (45×27×27 cm), with aluminium walls and opaque perspex floor, comprised two compartments the smaller (1/3) of which was painted black and the larger (2/3) white. The compartments were separated by a wooden partition with a centrally-positioned 7.5×7.5 cm opening at floor level, and the base of each compartment was marked with 9 cm square markings. Testing was performed in a room with standard background illumination. The white compartment was illuminated by three desk lamps (1450 lx) levels, while one red lamp was used to illuminate the dark compartment (30 lx). A videocamera, connected to a VCR and monitor in an adjacent laboratory, was positioned directly above the apparatus. The experiments were counterbalanced both for genetic strain and dose. After injection with vehicle or test compound, animals (n=10) were placed in small holding cages (17×7×6 cm) until testing. Thirty minutes later, they were individually placed in the centre of the white compartment and allowed 5 min exploration of the apparatus. The experimenter withdrew to an adjacent laboratory during testing and, between subjects, the apparatus was cleaned with damp and dry cloths.

Test videotapes were scored using the ethological software Hindsight (Weiss, 1995), and comprised both conventional and ethological measures (Holmes et al., 2001). In brief, the conventional measures were: inter-compartmental transitions; rearings; line crossings (LX) in each compartment and time spent in each compartment. The latter scores were used to derive the percentage of line crossings and time spent in the white compartment [i.e. %w scores=(white/total scores)×100] while

LX and time spent in each compartment were used to calculate rate scores for LX in each area (e.g. rate *W* LX). The principal ethological measures were the frequency of SAP and duration of immobility. Since percent immobility in the each compartment will be influenced by opportunity (time spent in that part of the apparatus), 'ratio time' scores were also calculated e.g. ratio black time for immobility (ratio *B* immobility = duration of immobility in black/time spent in black).

2.4.4. Vogel conflict (VCT)

This test was a modification of that described by Vogel et al. (1971) and recently used by Mathiasen and Mirza (2005). The test chambers (14.5 × 16 × 16 cm) were made from opaque white plastic and had metal grid floors. The spout of an externally-mounted water bottle protruded into the experimental chamber, and was positioned in the centre of one wall and 2.5 cm above the grid floor. This spout was connected to the shocker (Neuro-Search technical department). Experimental contingencies and measures (number of punished licks and latency to the 20th lick) were controlled and recorded by an IBM computer running in-house software. The light level in the room was 15 lx during habituation and test sessions. Mice were water deprived for 24 h in their home cage, following which they were permitted 6 min unpunished licking in the test chamber (habituation session). Only animals that completed more than 200 licks during the habituation session were included in the test session which commenced 4 h later. Mice (n = 8–30) were administered vehicle or test compound 30 min prior to the test session. Test sessions were identical to habituation sessions with the exception that every 20th lick resulted in a 0.14 mA, 500 ms electric shock through the water spout to the tongue. All sessions (habituation and test) started when mice were placed in the experimental chambers. Measures recorded were lick frequency and, to control for general behavioural suppression, the time taken to perform 20 licks (lick latency). Data for the effects of L-838,417, CDP and ZOL zolpidem on VCT performance in C57 mice have been published as part of a large-scale VCT validation study (Mathiasen and Mirza, 2005) and are reproduced here for completeness.

2.5. Statistical analysis

Dependent variables were analysed using either Statistica v. 4.5/7.1 (StatSoft Inc.) or SigmaStat v. 2.03 (SPSS Inc.). Prior to statistical analysis, all datasets were checked for normality and homogeneity of variance. Where necessary, individual datasets were transformed (e.g. 'square root') prior to further statistical treatment. Only a very small number of datasets proved resistant to transformation and, in these cases, results were analysed by non-parametric procedures (Kruskal–Wallis and Dunn's). With the exception of non-parametric datasets, all data were initially subjected to two-way independent ANOVA with strain (3 levels) and drug (4 levels) as factors. Significant interactions and significant main effects of strain were further explored using Newman–Keuls post-hoc comparisons. In view of the marked strain differences in behaviour, one-way independent ANOVAs (followed by Dunnett's comparisons) were then used to clarify drug effects (versus vehicle control) in each strain independently. It should be noted that, for the study on the effects of L-838,417 in the VCT, differing dose ranges were used for NMRI and DBA mice (3–30 mg/kg) compared to the C57 mice (1–10 mg/kg). As a two-way ANOVA analysis (strain × dose) was not possible, the effect of dose was analysed by a one-way ANOVA for each strain individually. In all cases, the critical alpha level was set at $P \leq 0.05$.

3. Results

3.1. Elevated plus-maze (EPM)

3.1.1. CDP

Results are summarised in Fig. 1. Significant main effects of strain were found for virtually all dependent variables [$F_{2,119} \geq 5.4$, $P \leq 0.01$]. On open arm avoidance measures (i.e. open entries, % open entries, % open time), DBA/2 mice showed the highest and C57 the lowest level of such anxiety-like behaviour. Significant strain × dose interactions were found for two measures: open arm entries and total head-dips [$F_{6,119} \geq 3.9$, $P \leq 0.001$]. In NMRI mice, 5–20 mg/kg CDP increased open arm entries ($P \leq 0.05$) and exerted a biphasic effect on total dips (increase at 5 mg/kg ($P < 0.02$); decrease at 20 mg/kg ($P < 0.002$). No significant effects of CDP on these parameters were observed in C57 mice whereas, in DBA mice, total dips were significantly increased at 10 mg/kg ($P < 0.05$). One-way ANOVAs revealed additional effects of CDP in each of the 3 strains.

NMRI. Fig. 1 (panels A and B). CDP significantly increased the total number of entries (5–10 mg/kg), % open arm entries (5–10 mg/kg) and % open arm time (5–20 mg/kg), while reducing % protected dips (5–20 mg/kg) [$F_{3,39} \geq 5.7$, $P \leq 0.005$]. In the interests of brevity, other datasets are not shown. However, consistent with its disinhibitory effect on time spent in the open arms, CDP concurrently reduced % closed time (5–10 mg/kg) and % mid time (10–20 mg/kg) [$F_{3,39} \geq 5.2$, $P \leq 0.005$]. No effects of treatment were found for total SAP [$F_{3,39} = 1.6$, NS], although a non-significant trend towards a reduction in % protected SAP was observed at all doses [$F_{3,39} = 2.1$, NS]. While rearing was decreased by 10–20 mg/kg CDP, this effect did not reach significance [$F_{3,39} = 2.5$, $P = 0.073$]. Importantly, the disinhibitory effects of CDP on open arm avoidance were observed in the absence of any change in closed arm entries (veh: 10.2 ± 0.9, 5 mg/kg: 10.9 ± 1.6, 10 mg/kg: 8.2 ± 1.9, 20 mg/kg: 10.9 ± 1.9; [$F_{3,39} = 0.6$, NS]). Despite this clear evidence for anxioselectivity, the already-noted reductions in total dips and protected dipping [$F_{3,39} \geq 12.6$, $P \leq 0.001$] suggest the possibility of a mild sedative action at the highest dose.

C57. Fig. 1 (panels C and D). Unlike the NMRI profile, C57 mice showed very few behavioural changes in response to CDP. In particular, drug treatment failed to alter % open arm entries, % open arm time or the % protected form of dips [$F_{3,39} \leq 1.9$, NS]. This general lack of effect on open arm avoidance is arguably due to the very low basal anxiety level characteristic of this strain. However, consistent with a sedative action, 20 mg/kg CDP significantly decreased both total arm entries and closed arm entries [$F_{3,39} \geq 2.9$, $P \leq 0.05$].

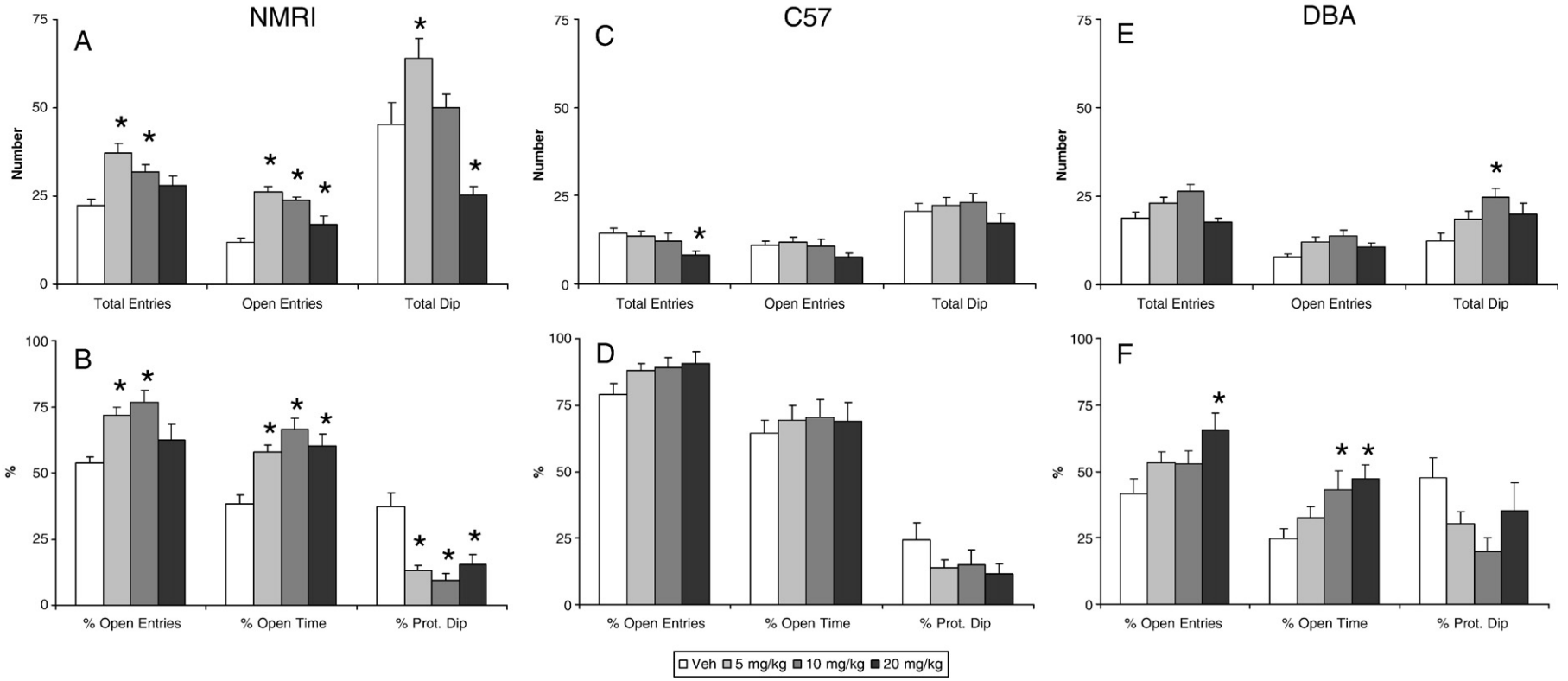

Fig. 1. The effects of CDP (5–20 mg/kg) in NMRI (panels A and B), C57 (panels C and D) and DBA (panels E and F) mice exposed to the elevated plus-maze (EPM). Panels A, C and E: total arm entries, open arm entries and total head-dips. Panels B, D and F: % open entries, % open time and % protected head-dips. Data are mean values ± SEM. See Tables 1 and 2 for corresponding data on L-838,417 and ZOL in all 3 strains, and text for further details. *$P \leq 0.05$ versus corresponding vehicle control.

Table 1
The effects of L-838,417 (3–30 mg/kg) in NMRI, C57 and DBA mice exposed to the elevated plus-maze (EPM)

	Vehicle	3 mg/kg	10 mg/kg	30 mg/kg
NMRI				
Total entries	18.6±0.8	23.0±2.3	24.6±1.4	22.8±1.7
Open entries	9.2±0.7	13.4±1.5	13.9±1.4*	13.9±1.1*
Total dip	33.7±4.1	36.1±4.0	40.2±6.9	30.1±4.1
Closed entries	9.3±0.5	9.6±1.2	10.7±0.9	8.9±1.1
Rearings	19.8±2.1	20.0±2.0	21.2±2.9	14.3±2.8
Total SAP	30.6±1.8	30.0±3.2	23.3±2.7	21.0±3.3
% Open entries	49.4±2.2	58.6±2.7	55.8±3.7	62.0±3.5
% Open time	37.9±4.0	43.1±3.2	44.3±4.1	47.1±4.3
% Prot. dip	33.8±7.9	34.5±5.3	30.4±7.0	20.2±3.2
% Closed time	30.8±2.0	25.8±2.4	26.7±1.5	26.2±3.1
% Mid time	31.3±4.3	31.2±1.9	29.0±3.1	26.6±2.1
% Prot. SAP	42.4±7.4	28.9±4.1	27.4±4.5	24.5±4.5
C57				
Total entries	15.3±1.2	17.7±1.8	18.2±1.1	15.3±1.2
Open entries	10.0±0.9	12.4±1.2	12.4±1.1	9.9±1.1
Total dip	14.8±2.1	14.9±0.7	17.7±2.3	15.3±1.8
Closed entries	5.3±0.9	5.2±1.3	5.8±0.7	5.4±0.7
Rearings	5.8±1.9	6.3±1.8	6.2±1.2	7.6±2.2
Total SAP	19.0±1.6	18.9±1.8	17.3±1.9	16.7±2.0
% Open entries	66.2±5.0	72.3±5.8	67.9±4.0	64.1±4.8
% Open time	45.9±6.1	49.6±4.8	49.0±2.8	44.1±4.2
% Prot. dip	34.9±6.5	32.0±4.4	43.0±7.3	39.3±7.9
% Closed time	20.0±3.7	17.7±4.9	19.2±2.5	20.9±2.7
% Mid time	34.1±3.8	32.6±2.5	31.8±1.7	35.1±3.4
% Prot. SAP	66.5±2.1	48.7±5.8*	46.7±5.6*	56.7±4.1
DBA				
Total entries	18.9±2.3	20.5±2.3	19.3±2.2	17.6±2.3
Open entries	7.3±1.7	7.3±1.0	8.7±1.3	7.9±1.3
Total dip	10.4±2.3	10.9±2.0	13.3±2.4	11.0±1.8
Closed entries	11.6±0.8	13.2±1.5	10.6±1.1	9.7±1.3
Rearings	16.9±1.7	17.1±1.6	12.9±0.8	9.9±1.9*
Total SAP	19.3±2.3	19.5±2.2	18.0±2.1	13.5±1.4
% Open entries	34.6±5.3	35.6±3.0	43.7±3.5	45.1±4.8
% Open time	20.9±3.8	19.8±2.2	26.3±3.5	24.7±4.6
% Prot. dip	43.5±8.2	52.0±6.4	33.5±6.2	41.0±5.9
% Closed time	42.6±4.4	45.8±2.4	40.2±4.0	36.2±3.7
% Mid time	36.5±2.5	34.4±2.0	33.5±1.9	39.1±4.1
% Prot. SAP	54.3±8.3	56.2±5.6	45.4±7.0	51.3±5.6

Data are mean values±SEM. See text for further details.
* $P \leq 0.05$ versus corresponding vehicle control.

DBA. Fig. 1 (panels E and F). CDP significantly increased % open arm entries (20 mg/kg) and % open arm time (10–20 mg/kg) [$F_{3,39} \geq 3.5$, $P \leq 0.05$]. Although the data are not shown, these changes were accompanied by a reduction in time spent in the closed arms [$F_{3,39}=4.9$, $P<0.01$] but no change in time spent on the central platform [$F_{3,39}=0.5$, NS]. The effects of CDP on open arm avoidance measures were observed in the absence of any significant change in either total arm entries [$F_{3,39}=1.5$, NS] or, more importantly, closed arm entries (veh: 11.0±1.4, 5 mg/kg: 11.0±1.6, 10 mg/kg: 12.6±2.5, 20 mg/kg: 7.0±2.5; [$H=5.8$, NS]). Despite some inhibitory trends, CDP did not alter total SAP or the % protected forms of SAP or dips [$F_{3,39} \leq 1.4$, NS]. Although this pattern supports an anxioselective action of CDP (10–20 mg/kg) in DBA mice, the possibility of high-dose muscle relaxation is suggested by the significant suppression of rear frequency [$F_{3,39}=4.9$, $P<0.01$].

3.1.2. L-838,417

Results are summarised in Table 1. Two-way ANOVA revealed significant main effects of strain for all dependent measures [$F_{2,114} \geq 4.9$, $P \leq 0.01$] except % protected head-dipping. Once again, the open arm avoidance indices confirmed a rank order of DBA>NMRI>C57 for anxiety-like behaviour. No significant strain × dose interactions were found [$F_{6,114} \leq 2.0$, NS], and one-way ANOVA were used to identify specific drug effects within each strain.

NMRI. Table 1 (upper). L-838, 417 (3–30 mg/kg) failed to alter any behavioural measure [$F_{3,39} \leq 2.8$, NS] with the sole

Table 2
The effects of zolpidem (0.3–3 mg/kg) in NMRI, C57 and DBA mice exposed to the elevated plus-maze (EPM)

	Vehicle	0.3 mg/kg	1 mg/kg	3 mg/kg
NMRI				
Total entries	15.2±1.4	16.4±1.2	13.4±1.6	9.4±1.2*
Open entries	8.3±1.2	8.8±0.8	7.6±1.1	5.6±0.9
Total dip	40.0±5.6	46.5±5.8	39.2±4.3	20.8±3.3*
Closed entries	6.9±0.8	7.6±0.6	5.8±0.9	3.8±0.5*
Rearings	12.6±1.7	17.3±1.6	10.9±1.9	5.3±1.6*
Total SAP	30.0±1.6	28.5±2.0	21.6±2.1*	11.3±1.6*
% Open entries	54.3±5.1	53.3±2.4	57.9±6.5	57.1±6.9
% Open time	39.7±5.7	38.4±2.9	43.3±6.1	37.8±7.7
% Prot. dip	38.9±6.1	37.1±5.5	35.8±9.3	33.4±8.6
% Closed time	23.4±3.0	25.6±1.5	23.1±3.4	38.1±7.6
% Mid time	36.9±3.4	36.0±3.4	33.6±4.6	24.1±4.2
% Prot. SAP	40.0±5.2	39.3±5.9	30.9±8.4	36.9±9.6
C57				
Total entries	14.8±1.1	14.0±0.7	12.2±0.7	1.5±0.8*
Open entries	12.0±0.6	11.4±0.4	8.6±0.7	1.2±0.6*
Total dip	27.8±3.4	25.9±1.9	20.9±1.7	4.4±1.9*
Closed entries	2.8±0.7	2.6±0.6	3.6±0.9	0.3±0.2*
Rearings	2.3±0.9	1.5±0.5	2.6±0.9	0.0±0.0
Total SAP	19.8±1.3	22.7±2.2	17.2±2.1	3.6±1.1*
% Open entries	82.6±3.6	82.5±3.5	72.3±6.2	75.0±13.7
% Open time	61.0±2.8	59.8±3.4	47.0±6.1	20.3±12.3*
% Prot. dip	22.7±2.8	27.1±3.6	31.0±6.5	66.0±11.0*
% Closed time	8.9±2.3	8.4±2.2	17.8±4.5	9.3±7.9
% Mid time	30.1±2.3	31.7±2.7	35.2±3.4	70.4±13.2
% Prot. SAP	52.2±5.1	52.0±5.0	59.1±7.2	72.5±13.2
DBA				
Total entries	15.2±2.2	16.4±1.7	5.4±0.8*	3.7±1.7*
Open entries	4.9±0.8	7.1±0.7*	2.7±0.5*	1.8±0.8*
Total dip	11.9±2.0	14.6±2.5	5.9±1.3*	3.5±1.1*
Closed entries	10.3±1.5	9.3±1.5	2.7±0.6*	1.9±1.1*
Rearings	14.6±2.1	15.1±1.8	2.1±0.4	1.5±1.1
Total SAP	16.8±1.9	22.3±2.8	11.8±1.8	8.3±3.1*
% Open entries	29.4±4.2	45.9±5.2	54.1±10.7	50.6±10.9
% Open time	15.3±2.9	24.3±5.1	24.3±7.5	9.7±4.9
% Prot. dip	63.2±6.1	41.1±6.5	62.1±9.5	58.7±12.2
% Closed time	51.4±4.8	38.1±4.9	28.9±8.3	23.0±8.4*
% Mid time	33.3±2.4	37.6±3.3	46.9±6.3	67.3±11.1
% Prot. SAP	65.2±7.4	43.1±7.5	67.1±6.7	71.9±9.7

Data are mean values±SEM. See text for further details.
* $P \leq 0.05$ versus corresponding vehicle control.

exception of a significant increase in the number of open arm entries at 10–30 mg/kg [$F_{3,39}=3.4$, $P<0.05$]. As no other open arm measures were significantly altered, this isolated effect of L-838,417 in NMRI mice cannot be interpreted as evidence of an anxiolytic-like action. This finding stands in marked contrast to the potent anxiolytic profile of CDP in the same strain. *C57*. Table 1 (centre). Over the dose range tested, L-838,417 also had minimal behavioural effects in C57 mice [$F_{3,39} \leq 2.0$, NS; $H(3) \leq 3.1$, NS]. Indeed, the only significant behavioural change observed was a reduction in % protected SAP at doses of 3–10 mg/kg [$F_{3,39}=3.6$, $P<0.05$]. As was the case for CDP, the lack of effect of L-838,417 in C57 mice may well have be due to the low anxiety baseline. *DBA*. Table 1 (lower). Despite the high anxiety baseline in DBA mice (relative to NMRI and C57), L-838,417 (3–30 mg/kg) was again largely devoid of significant behavioural activity [$F_{3,39} \leq 2.0$, NS; $H(3) \leq 6.8$, NS]. The sole exception was a significant reduction in rear frequency at 30 mg/kg [$F_{3,39}=5.0$, $P<0.01$].

3.1.3. ZOL

Results are summarised in Table 2. Two-way ANOVA again found significant main effects for strain on all measures [$F_{2,114} \geq 6.5$, $P \leq 0.005$], with a rank order of DBA > NMRI ≥ C57 for the principal measures of anxiety-like behaviour. Significant interactions were found for rear frequency, number of open arm entries, total dips and % protected dips [$F_{6,114} \geq 2.2$, $P \leq 0.05$]. Post-hoc analysis showed that, in NMRI mice, 3 mg/kg ZOL decreased

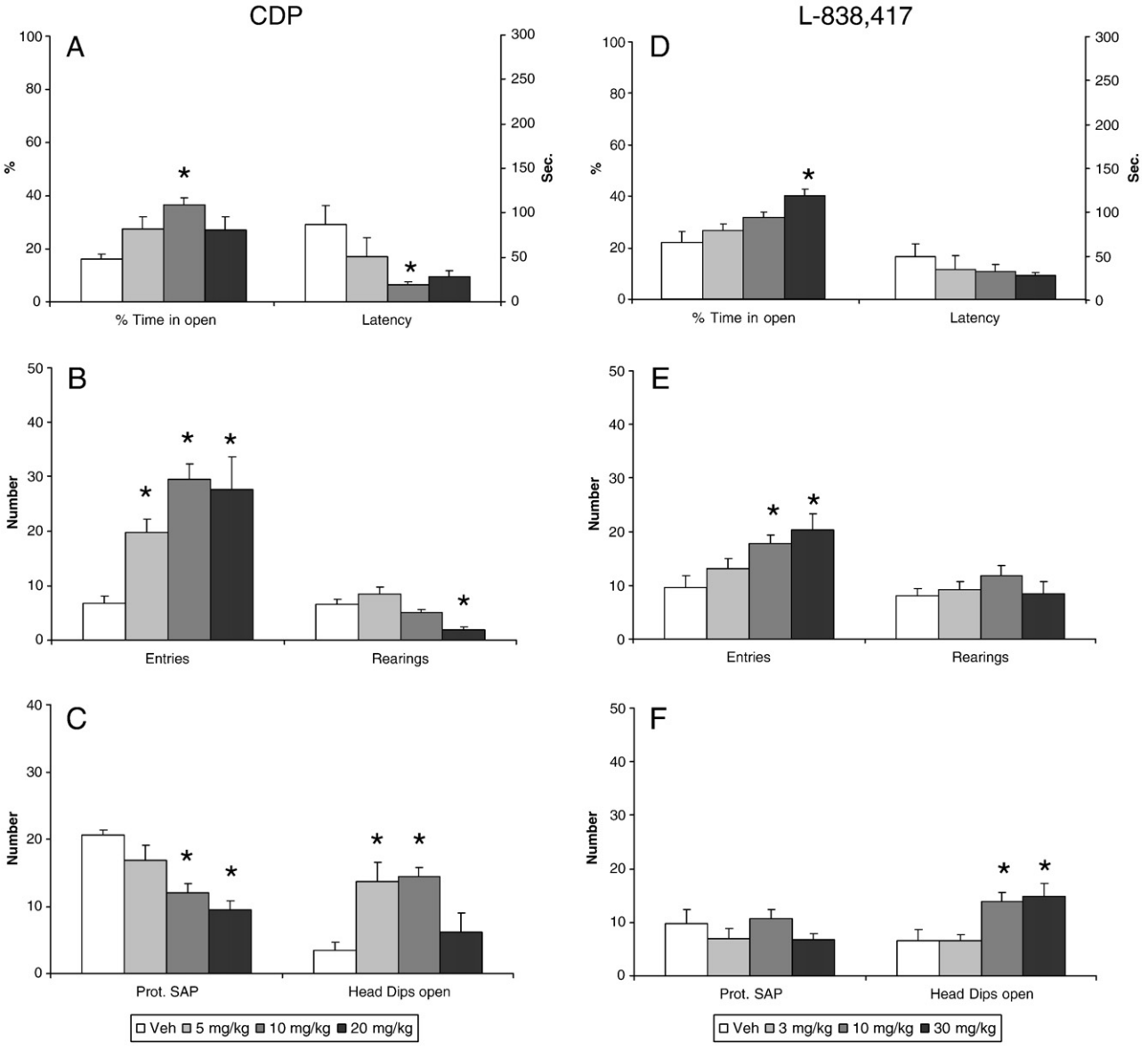

Fig. 2. The effects of CDP (5–20 mg/kg; panels A–C) and L-838,417 (3–30 mg/kg; panels D–F) in NMRI mice exposed to the elevated zero-maze (ZM). Panels A and D: % time on open quadrants and latency to enter open quadrant. Panels B and E: total number of open entries and total rearings. Panels C and F: number of protected SAP and head-dips on open quadrants. Data are mean values ± SEM. See Table 3 for corresponding data on C57 and DBA strains, and text for further details. *$P \leq 0.05$ versus corresponding vehicle control.

rearing and total dips ($P \leq 0.001$); in C57 mice, 3 mg/kg decreased open arm entries, total dips and % protected dips ($P \leq 0.01$); and, in DBA mice, 1–3 mg/kg decreased rearings and total dips ($P \leq 0.05$). Interestingly, a biphasic effect of ZOL on open arm entries was observed in this strain, with an increase at 0.1 mg/kg ($P<0.05$) and reductions at 1–3 mg/kg ($P \leq 0.04$). Additional (mainly, sedative-like) effects of ZOL were revealed by a series of one-way ANOVA.

NMRI. Table 2 (upper). ZOL (3 mg/kg) significantly reduced total and closed arm entries [$F_{3,39}>5.0$, $P<0.005$]. Furthermore, 1–3 mg/kg ZOL significantly reduced the total number of SAPs [$F_{3,39}=21.5$, $P<0.005$]. These data, together with the two-way ANOVA findings reviewed above, indicate that the highest dose of ZOL had a clear sedative effect in NMRI mice. *C57*. Table 2 (centre). ZOL (3 mg/kg) significantly decreased total and closed arm entries, total SAP and % open arm time [$F_{3,39} \geq 24.1$, $P \leq 0.001$; $H(3) \geq 9.5$, $P \leq 0.05$]. This profile is again indicative of a sedative effect. *DBA*. Table 2 (lower). ZOL (3 mg/kg) significantly reduced total and closed arm entries, total SAP, % open arm entries and % closed arm time [$F_{3,39} \geq 3.3$, $P \leq 0.05$; $H(3) \geq 8.1$, $P \leq 0.05$], again confirming a sedative-like action of the compound. Indeed, it appears that DBA mice were more sensitive than the other strains to the sedative action of ZOL, e.g. inhibitory effect of 1 mg/kg on total and closed arm entries ($P \leq 0.05$).

3.2. Elevated zero-maze (ZM)

3.2.1. CDP

Findings are summarised in Fig. 2 and Table 3 (upper). Following transformation, only 3 of the 6 dependent measures complied with normality and homogeneity of variance criteria. For these 3 measures, two-way ANOVA found main effects of strain for rearings, open entries and protected SAP [$F_{2,88} \geq 3.1$, $P \leq 0.05$] as well as a significant strain × dose interaction for open entries [$F_{6,88}=6.5$, $P<0.001$]. Post-hoc tests revealed that vehicle-treated C57 animals made significantly fewer entries into the open areas compared to the NMRI and DBA strains ($P \leq 0.05$), indicating a rank order of C57>NMRI=DBA for anxiety-like behaviour in the ZM. Post-hoc analysis also showed that all doses of CDP significantly increased open entries ($P \leq 0.001$) but only in NMRI mice.

NMRI. Fig. 2 (panels A–C). One-way ANOVA demonstrated that CDP increased time spent in the open areas (10 mg/kg) and the frequency of open dips (5–10 mg/kg), while simultaneously decreasing the latency to enter an open area (10 mg/kg), the frequency of protected SAP (10–20 mg/kg) and total rearings (20 mg/kg) [$F_{3,27} \geq 4.1$, $P \leq 0.02$]. Although the high-dose reduction in rearing might suggest sedation, this effect would be a logical consequence of increased activity in the open quadrants. Overall, therefore, the ZM profile of CDP in NMRI mice is consistent with a robust anxiolytic effect. *C57*. Table 3 (upper). Despite a very high basal level of anxiety, CDP was without anxiolytic-like activity in C57 mice [$F_{3,26} \leq 2.6$, NS]. Indeed, 20 mg/kg significantly reduced time in open, frequency of SAP and rearing, whereas 5 mg/kg CDP increased the frequency of rearings [$F_{3,31} \geq 4.0$, $P \leq 0.02$; $H(3) \geq 9.2$, $P \leq 0.03$]. *DBA*. Table 3 (upper). CDP did not have a consistent anxiolytic profile in this strain, the sole exception being a reduction in the frequency of protected SAP at 20 mg/kg [$F_{3,28}=10.0$, $P<0.001$]. In the absence of other significant behavioural changes, it could be argued that this selective effect of CDP reflects a weak anxiolytic-like action.

Table 3
The effects of CDP (5–20 mg/kg; panel A) and L-838,417 (3–30 mg/kg; panel B) in C57 and DBA mice exposed to the elevated zero-maze (ZM)

A

	Vehicle	5 mg/kg	10 mg/kg	20 mg/kg
C57				
% Open time	3.9±1.2	3.9±1.2	1.5±0.6	0.5±0.4*
Latency	149.9 ±44.0	143.4±42.0	221.8±38.6	258.9±31.0
Entries	1.9±0.7	2.6±0.8	0.9±0.4	0.1±0.1
Rearings	3.6±0.8	6.3±0.9*	2.4±0.6	0.4±0.2*
Prot. SAP	17.8±1.8	19.4±2.3	13.6±1.8	7.6±1.2*
Dip open	0.3±0.2	1.1±0.5	1.1±0.5	0.0±0.0
DBA				
% Open time	22.6±4.5	19.9±4.4	14.7±4.9	17.4±8.8
Latency	104.6±31.5	57.9±11.5	136.1±48.4	165.5±61.1
Entries	7.4±1.9	6.3±0.8	6.0±2.3	6.5±4.2
Rearings	9.6±2.3	11.4±2.3	8.7±1.1	3.7±1.1
Prot. SAP	16.4±1.8	14.3±1.9	15.3±1.8	3.5±1.4*
Dip open	1.1±0.4	3.4±0.6	5.1±2.8	8.5±4.1

B

	Vehicle	3 mg/kg	10 mg/kg	30 mg/kg
C57				
% Open time	6.8±1.5	7.3±2.4	9.0±2.7	10.7±3.3
Latency	105.0±28.4	105.8±37.3	90.0 ±32.2	77.4±33.6
Entries	3.6±0.7	3.8±1.1	6.3±2.0	4.9±1.3
Rearings	12.2±1.3	9.3±0.9	14.5±0.8	7.5±1.2*
Prot. SAP	13.9±1.8	10.8±1.3	12.8±1.4	11.0±2.3
Dip open	1.1±0.4	2.3±1.1	2.4±0.7	2.1±0.8
DBA				
% Open time	33.7±9.9	23.4±5.8	23.2±5.9	29.6±5.3
Latency	74.8±23.7	115.6±37.5	120.1±24.6	76.6±12.9
Entries	5.6±1.6	6.9±2.0	6.8±2.0	10.4±2.1
Rearings	8.1±1.3	6.8±0.6	10.8±2.1	7.5±1.5
Prot. SAP	11.1±2.3	11.3±1.3	8.1±1.2	9.0±2.6
Dip open	6.1±2.4	2.5±1.1	4.5±2.1	10.1±1.9

See Fig. 2 for corresponding datasets on the NMRI strain, and text for further details. Data are mean values±SEM.
* $P \leq 0.05$ versus corresponding vehicle control.

3.2.2. L-838,417

Data are summarised in Fig. 2 and Table 3 (lower). Two-way ANOVA found significant main effects of strain for all dependent measures [$F_{2,97} \geq 3.4$, $P \leq 0.05$] except for latency to enter an open area. Once again, based on time spent in the open quadrants, C57 mice seemed the most anxious of the 3 strains tested. However, there were no significant drug × strain interactions [$F_{6,97} \leq 2.0$, NS]. One-way ANOVA were used to further explore the effects of L-838,417 in each strain.

NMRI. Fig. 2 (panels D–F). Consistent with an anxiolytic-like effect, one-way ANOVA found significant increases in

percent time in open (30 mg/kg), number of open entries and open dips (10–30 mg/kg) [$F_{3,31} \geq 4.8$, $P \leq 0.01$]. C57. As summarised in Table 3 (lower), and despite high basal anxiety levels, L-838,417 (3–30 mg/kg) was without anxiolytic effect in the C57 strain [$F_{3,39} \leq 0.9$, NS]. Nevertheless, the drug was not completely without effect in these animals, decreasing the frequency of rearing at 30 mg/kg [$F_{3,33}=7.7$, $P<0.001$]. *DBA*. Table 3 (lower). L-838,417 (3–30 mg/kg) was without significant effect any of the dependent measures [$F_{3,31} \leq 2.8$, NS].

3.2.3. ZOL

Results are summarised in Table 4. Two-way ANOVA found significant main effects of strain for all measures [$F_{2,103} \geq 3.7$, $P \leq 0.05$], together with strain × dose interactions for latency, rears, and protected SAP [$F_{6,103} \geq 3.2$, $P \leq 0.01$]. Post-hoc analysis revealed that vehicle-treated C57 mice had lower scores for latency, rears and protected SAP ($P \leq 0.01$) compared to control animals of the NMRI and DBA strains. Furthermore, in NMRI mice, 3 mg/kg ZOL significantly reduced rearing ($P<0.001$). In C57 mice, the 3 mg/kg dose significantly increased latency, and decreased both rearing and the frequency of protected SAP ($P \leq 0.05$). In DBA mice, rearings and protected SAP were reduced at both 1 and 3 mg/kg ($P \leq 0.001$).

NMRI. Table 4 (upper). One-way ANOVA on the remaining measures failed to find further significant effects of this compound [$F_{3,31} \leq 1.5$, NS] over and beyond the already identified inhibition of rearing at 3 mg/kg. *C57*. Table 4 (centre). 3 mg/kg ZOL significantly decreased time in the open while 1–

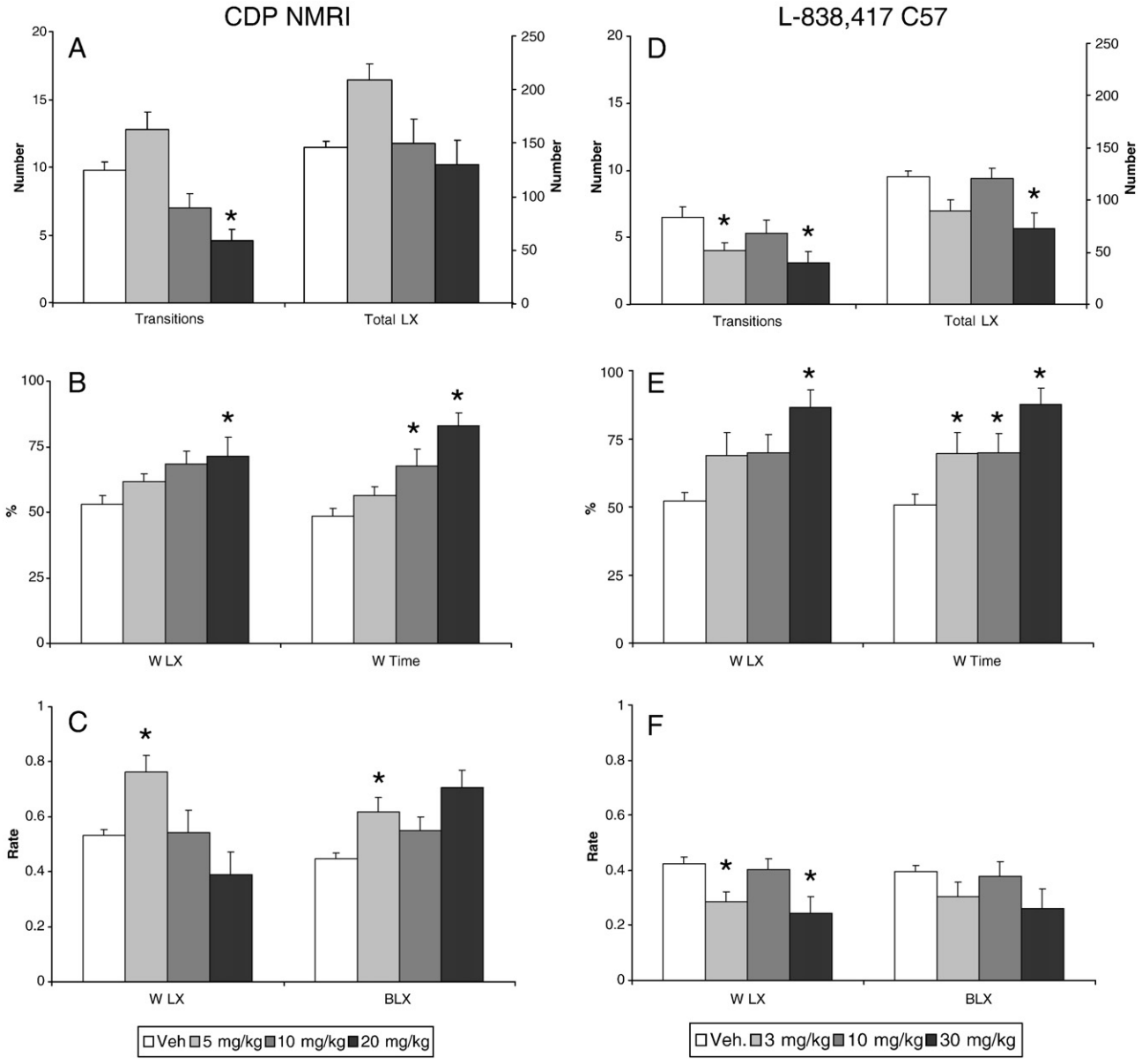

Fig. 3. The effects of CDP (5–20 mg/kg) in NMRI mice (panels A–C) and of L-838,417 (3–30 mg/kg) in C57 mice (panels D–F) exposed to the light/dark exploration test (LDE). Panels A and D: total transitions and total line crosses (LX). Panels B and E: percent line crosses in white (%wLX) and percent time spent in white (%w time). Panels C and F: rate of line crosses in the white ` and rate of line crosses in the black. Data are mean values ± SEM. See Tables 5–7 for complementary data, and text for further details. *$P \leq 0.05$ versus corresponding vehicle control.

Table 4
The effects of zolpidem (0.3–3 mg/kg) in NMRI, C57 and DBA mice exposed to the elevated zero-maze (ZM)

	Vehicle	0.3 mg/kg	1 mg/kg	3 mg/kg
NMRI				
% Open time	17.6±4.7	26.3±4.3	17.7±4.0	13.2±6.1
Latency	130.1±43.3	43.1±9.0	27.4±4.5	91.9±45.8
Entries	6.8±1.7	9.7±0.8	7.3±2.0	3.6±1.6
Rearings	7.3±1.5	5.9±0.9	3.9±1.1	1.1±0.3 *
Prot. SAP	13.9±2.4	10.5±1.8	10.6±1.8	8.0±1.4
Dip open	8.6±3.0	10.6±2.1	6.1±1.2	6.1±2.8
C57				
% Open time	31.7±6.5	25.7±4.9	18.0±6.5	5.7±5.7 *
Latency	21.8±5.2	20.3±3.9	51.9±23.1	229.5±70.5 *
Entries	8.3±0.7	9.6±0.9	5.2±1.0 *	0.3±0.3 *
Rearings	3.7±0.8	4.4±0.6	4.1±0.9	0.0±0.0 *
Prot. SAP	5.7±1.0	4.0±1.0	5.0±1.0	0.8±0.3 *
Dip open	6.8±1.6	4.9±1.3	2.8±1.1	0.5±0.5
DBA				
% Open time	9.6±3.9	20.5±6.1	12.5±6.1	0.0±0.0
Latency	139.9±38.5	107.0±43.0	217.6±44.5	300.0±0.0
Entries	3.3±1.4	5.1±1.4	0.6±0.4	0.0±0.0 *
Rearings	8.0±1.5	9.6±1.3	1.4±0.5 *	0.0±0.0 *
Prot. SAP	12.1±1.8	9.9±1.5	3.0±0.8 *	0.1±0.1 *
Dip open	1.4±0.7	4.9±1.6	1.3±0.7	0.0±0.0

Data are mean values±SEM. See text for further details.
* $P \leq 0.05$ versus corresponding vehicle control.

3 mg/kg reduced the number of open entries [$F_{3,39} \geq 4.3$, $P \leq 0.005$]. *DBA*. Table 4 (lower). 3 mg/kg ZOL decreased the number of open entries [$F_{3,31} = 6.9$, $P = 0.001$].

3.3. Light/dark exploration (LDE)

3.3.1. CDP

Data are summarised in Fig. 3 (panels A–C) and Table 5. Two-way ANOVA revealed significant main effects of strain on all dependent measures [$F_{2,117} \geq 3.4$, $P \leq 0.05$], with a strain rank order of DBA > NMRI ≥ C57 for the principal measures of anxiety-like behaviour (i.e. avoidance of the brightly-lit compartment). Only one significant strain × dose interaction [$F_{6,117} = 4.1$, $P < 0.001$] was found, with post-hoc tests indicating that 20 mg/kg CDP significantly decreased the rate of black LX in C57 and DBA mice ($P \leq 0.008$).

NMRI. Fig. 3 (panels A–C) and Table 5 (upper). One-way ANOVA demonstrated significant treatment effects on transitions, total LX, total rearing, total immobility, %wLX, %w time, rate of white LX, and ratio white time immobility [$F_{3,39} \geq 3.1$, $P \leq 0.05$; $H(3) \geq 12.8$, $P \leq 0.005$]. Although post-hoc comparisons showed that CDP was without significant effect on total LX over the dose range tested, significant increases in the rate of LX in both the black and white compartments at 5 mg/kg ($P \leq 0.005$) suggested a general stimulant effect at this low dose level. At 10 mg/kg, CDP significantly ($P \leq 0.05$) increased the %w scores for time, a profile consistent with anxiolytic activity. Although significant increases in this score (plus %wLX) were observed at 20 mg/kg ($P \leq 0.03$), this dose concurrently reduced transitions and total rearing ($P \leq 0.05$) while increasing both total immobility and ratio white time immobility ($P \leq 0.05$). CDP (10–20 mg/kg) produced many behavioural changes indicative of reduced anxiety in NMRI mice. While some of the behavioural effects (e.g. increased immobility) observed at 20 mg/kg might suggest concomitant sedation, the lack of effect of this dose both on total LX and the rate of LX (both compartments) would argue against behavioural non-specificity.

C57. Table 5 (centre). CDP treatment had significant effects on transitions, total LX, total rearing, total immobility, %wLX, %w time, rate of white LX, total rearing, total immobility, ratio white time immobility and ratio black time immobility [$F_{3,39} \geq 2.9$, $P \leq 0.05$; $H(3) \geq 23.9$, $P \leq 0.001$]. Post-hoc tests showed that 10 mg/kg CDP significantly increased %wLX, total immobility and the ratio time measure for immobility in the white compartment ($P \leq 0.05$). Although the highest dose (20 mg/kg) significantly increased %w scores for LX and time, this anxiolytic-like profile was associated with substantial reductions in transitions, total rears, and total LX, as well as reduced rates of LX in both compartments, increased total immobility, and increased ratio time measures for immobility in both compartments ($P \leq 0.05$). Despite evidence consistent with anxiolytic-like activity, CDP predominantly exerted a sedative-like action in C57 mice.

Table 5
The effects of CDP (5–20 mg/kg) in NMRI, C57 and DBA mice exposed to the light/dark exploration test (LDE)

	Vehicle	5 mg/kg	10 mg/kg	20 mg/kg
NMRI				
Rearings	55.1±3.7	57.6±4.9	37.2±8.0	19.4±5.7 *
Total SAP	12.1±3.6	8.4±1.6	5.5±1.4	3.4±1.2
Total immobility (s)	2.4±1.2	2.7±2.3	52.2±22.8	119.2±18.6 *
Ratio *W* immobility	0.0±0.0	0.0±0.0	0.2±0.1	0.5±0.1 *
Ratio *B* immobility	0.0±0.0	0.0±0.0	0.1±0.0	0.0±0.0
C57				
Transitions	6.1±1.1	5.2±0.9	4.5±1	2.33±0.62 *
Rearings	33.6±5.3	24.4±3.8	23.2±4.5	3.78±1.36 *
Total SAP	11.9±1.4	18.6±1.8	12.9±1.8	9.67±2.98
Total immobility (s)	9.22±5.5	19±5.4	40.5±17 *	119±16.8 *
Total LX	127±13	119±9.7	120±17	68.2±12 *
%wLX	59.8±7.8	73.2±5.6	68.9±6.1 *	85.1±6.07 *
%w time	59±7.8	76.7±5.1	72.5±6.2	88.7±4.86 *
Rate *W* LX	0.43±0	0.38±0	0.38±0.1	0.22±0.04 *
Rate *B* LX	0.47±0	0.47±0	0.55±0	0.34±0.02 *
Ratio *W* immobility	0.03±0	0.07±0	0.15±0.1 *	0.42±0.06 *
Ratio *B* immobility	0±0	0.02±0	0.02±0	0.22±0.05 *
DBA				
Transitions	3.1±0.7	5.4±1.2	3.3±0.5	2.7±0.47
Rearings	39.2±1.7	44.7±7.4	20.1±6.6 *	4.8±1.26 *
Total SAP	16.9±3.3	10.9±1.4	13.3±1.8	7±0.79 *
Total immobility (s)	7.97±2.8	22±17	31.3±9.9	104±17.3 *
Total LX	135±7.3	162±21	116±21	62.4±9.04 *
%wLX	15.2±3.4	32.4±7.1	35.8±4.4 *	50.6±9.94 *
%w time	24±3.1	38.5±7.5	34.9±4.2	46.1±9.65
Rate *W* LX	0.26±0	0.45±0.1	0.42±0.1	0.27±0.06
Rate *B* LX	0.5±0	0.62±0	0.38±0.1	0.22±0.04 *
Ratio *W* immobility	0.02±0	0.08±0.1	0.02±0	0.16±0.09
Ratio *B* immobility	0.03±0	0.01±0	0.15±0	0.39±0.08 *

Data are mean values±SEM. See Fig. 3 for complementary data on the NMRI strain, and text for further details.
* $P \leq 0.05$ versus corresponding vehicle control.

DBA. Table 5 (lower). In addition to effects already described above, treatment had significant effects on total LX, total rears, total SAP, total immobility, %wLX and ratio black time immobility [$F_{3,39} \geq 4.2$, $P \leq 0.02$; $H(3) \geq 16.4$, $P \leq 0.001$]. Post-hoc analyses revealed that, at 10–20 mg/kg, CDP significantly increased %wLX ($P \leq 0.05$), a profile consistent with anxiolytic activity. However, the high-dose effects were accompanied by decreased total scores for LX, rearing (also seen at 10 mg/kg) and SAP, as well as a substantial increase in total immobility ($P \leq 0.02$). A sedative effect of the highest dose was further supported by a significant reduction in the rate of LX and increased ratio time immobility in the black compartment ($P \leq 0.05$). Although the data indicate weak anxiolytic activity of CDP (10 mg/kg) in DBA mice, the profile of 20 mg/kg CDP was strongly suggestive of sedation. In this respect, and despite major differences in behavioural baselines, clear parallels can be drawn between the effects of CDP in C57 and DBA mice.

3.3.2. L-838,417

Data are summarised in Fig. 3 (panels D–F) and Table 6. Two-way ANOVA found significant main effects of strain [$F_{2,116} \geq 8.5$, $P \leq 0.001$] on 6 behavioural measures, again consistent with an anxiety rank ordering of DBA ≥ NMRI ≥ C57. Strain × dose interactions were significant for total LX, total rearing and %w time [$F_{6,116} \geq 2.8$, $P \leq 0.05$] and these were further explored by Newman–Keuls post-hoc test. Vehicle control subjects from each strain differed significantly in terms of %w time ($P \leq 0.005$), with DBA < NMRI = C57. Post-hoc analysis further revealed that, in NMRI mice, rearing was significantly decreased by L-838,417 at doses of 10–30 mg/kg ($P \leq 0.05$). In C57 mice, all doses of L-838,417 significantly increased %w time ($P \leq 0.05$), rearing was reduced at doses of 3 and 30 mg/kg, and LX at 30 mg/kg ($P \leq 0.05$). In DBA mice, total LX were significantly increased by 3 mg/kg ($P = 0.028$) while rearing was decreased by 10–30 mg/kg ($P \leq 0.05$).

NMRI. Table 6 (upper). One-way ANOVA additionally indicated that L-838,417 treatment significantly influenced total immobility [$H(3) = 9.2$, $P < 0.05$], this effect being attributable to a modest increase at the highest dose tested ($P < 0.05$). Despite the changes in rearing (see above) and immobility, the compound was without effect on transitions, total SAP and LX, the rate measures for LX in both compartments, and both %w scores [$F_{3,39} \leq 1.5$, NS; $H(3) \leq 3.2$, NS]. This profile indicates that L-838,417 was devoid of anxiolytic-like activity in NMRI mice over the dose range tested, although there was some evidence for a muscle-relaxant (rather than sedative) action at 10–30 mg/kg.

C57. Fig. 3 (panels D–F) and Table 6 (centre). In direct contrast to its fairly minimal effects in the NMRI strain, L-838,417 was effective in altering a large range of LDE measures in C57 mice. In addition to effects already described, L838,417 significantly altered transitions, %wLX, rate *W* LX and ratio white immobility time [$F_{3,39} \geq 3.4$, $P \leq 0.05$; $H(3) \geq 18.7$, $P \leq 0.001$]. Post-hoc tests showed that L-838,417 increased %wLX (30 mg/kg) and reduced total rears (3 and 30 mg/kg) ($P \leq 0.05$). Furthermore, paralleling the unusual dose-response pattern observed for total rearing (see above), 3 and 30 mg/kg L-838,417 reduced transitions and the rates of LX in the white (but not black) compartment ($P \leq 0.05$); these doses also increased the ratio time score for immobility in the white (but not black) compartment ($P < 0.05$). Somewhat curiously, 3 and 30 mg/kg L-838,417 appeared to exert a combination of anxiolysis and sedation/muscle relaxation in C57 mice whereas the intermediate dose of 10 mg/kg induced a clear anxiolytic profile.

Table 6
The effects of L-838,417 (3–30 mg/kg) in NMRI, C57 and DBA mice exposed to the light/dark exploration test (LDE)

	Vehicle	3 mg/kg	10 mg/kg	30 mg/kg
NMRI				
Transitions	8.3±1	10.3±0.8	8.8±0.8	8±1.35
Rearings	52.9±6	58.2±5.2	39.8±3.7*	25.9±6.33*
Total SAP	10.7±3.1	14.4±3.9	9±1.7	9.56±2.15
Total immobility (s)	0.49±0.3	1.04±0.5	8.13±5.3	28.4±14*
Total LX	126±14	150±9.7	136±13	119±14.7
%wLX	55.9±4.4	48±5.8	54.1±4.6	56.8±7.77
%w time	51.9±5.8	44.5±6.2	50.8±6	55.8±7.9
Rate *W* LX	0.47±0.1	0.56±0	0.51±0.1	0.4±0.06
Rate *B* LX	0.41±0	0.47±0	0.43±0	0.41±0.05
Ratio *W* immobility	0±0	0±0	0.03±0	0.1±0.05
Ratio *B* immobility	0±0	0±0	0.02±0	0.02±0.01
C57				
Rearings	42.4±3.1	26.9±4.3*	40.5±3.3	14.3±3.7*
Total SAP	13.6±2.2	20.6±2.2*	14.4±1.6*	20.9±2.7*
Total immobility (s)	2.2±0.8	23.2±6.8	14.5±4.7	64.3±14.1
Ratio *W* immobility	0.0±0.0	0.1±0.0*	0.1±0.0	0.2±0.0*
Ratio *B* immobility	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
DBA				
Transitions	4±0.6	3.4±0.8	2.3±0.3	3±1.19
Rearings	44.1±3.6	55.8±4.1	30±5.6*	23.2±4.64*
Total SAP	17±2.2	16±2.5	16.3±2.8	13.8±2.54
Total immobility (s)	1.12±0.6	1.6±1	5.62±2.6	13.7±8.35
Total LX	137±7.6	176±9.8*	122±16	116±19.1
%wLX	17.2±2.4	17.4±4.9	13.7±1.9	14.4±3.34
%w time	26.4±3.2	27.1±5	24.2±1.7	24.5±3.29
Rate *W* LX	0.3±0	0.35±0.1	0.22±0	0.22±0.06
Rate *B* LX	0.52±0	0.67±0	0.47±0.1	0.44±0.07
Ratio *W* immobility	0±0	0±0	0±0	0.06±0.06
Ratio *B* immobility	0±0	0±0	0.02±0	0.03±0.03

Data are mean values±SEM. See Fig. 3 for complementary data on the C57 strain, and text for further details.
* $P \leq 0.05$ versus corresponding vehicle control.

DBA. Table 6 (lower). As in previous experiments, DBA mice showed a higher basal level of anxiety-like behaviour than did either NMRI or C57 mice (e.g. %w scores for LX and time). Although detection of anxiolytic-like activity would theoretically be favoured by a high baseline, L-838,417 did not significantly alter the major indices of anxiety in this strain, i.e. apart from the already-noted inhibitory effects on total rearing (10–30 mg/kg), one-way ANOVA found no significant effects of L-838,417 in DBA mice [$F_{3,39} \leq 1.0$, NS; $H(3) \leq 5.8$, NS]. As the substantial reductions in rearing were not associated with significant decreases in other active behaviours, some non-specific effect other than sedation seems likely.

3.3.3. ZOL

Findings are summarised in Table 7. Two-way ANOVA analysis revealed significant main effects of strain for total

Table 7
The effects of zolpidem (0.3–3 mg/kg) in NMRI, C57 and DBA mice exposed to the light/dark exploration test (LDE)

	Vehicle	0.3 mg/kg	1 mg/kg	3 mg/kg
NMRI				
Transitions	8.78±0.9	7.88±0.9	8±0.9	2±0.33*
Rearings	51.8±5	52.6±5.5	33.7±5.1	5.33±1.95*
Total SAP	27.8±5	13.5±4.1*	15.2±2.3*	6±2.03*
Total immobility (s)	0.24±0.2	0.87±0.8	4.87±2.9	119±25.3*
Total LX	111±7.8	120±7.6	93.5±8.7	36.3±9.16*
%wLX	56.5±3.9	53±4.4	55±5.7	64.9±13.4
%w time	52.2±3.8	50.7±5	53±7	64.7±16.7*
Rate *W* LX	0.4±0	0.42±0	0.34±0	0.15±0.02*
Rate *B* LX	0.34±0	0.39±0	0.3±0	0.25±0.09
Ratio *W* immobility	0±0	0±0	0.02±0	0.24±0.1
Ratio *B* immobility	0±0	0±0	0.01±0	0.26±0.12
C57				
Transitions	7.1±1	5.8±0.7	4.4±0.6	1.7±0.23*
Rearings	47±3	35.6±3	31.8±7.1	3.6±1.68*
Total SAP	14.1±2	14.4±1.8	14±3.2	14.1±2.5
Total immobility (s)	2.58±1.8	4.59±1.3	21.3±7.3*	139±15.8*
Total LX	130±9.2	115±6.8	87.3±9.6*	21.8±4.4*
%wLX	57.6±6.3	54.2±7.5	53.1±7.9	52.8±12.4
%w time	55.8±6.5	52.9±7.4	50.4±9.2	59.1±11.1
Rate *W* LX	0.45±0	0.4±0	0.34±0	0.08±0.02*
Rate *B* LX	0.41±0	0.38±0	0.3±0*	0.08±0.01*
Ratio *W* immobility	0.01±0	0.02±0	0.06±0	0.43±0.08*
Ratio *B* immobility	0±0	0.01±0	0.04±0*	0.4±0.07*
DBA				
Transitions	3.7±1.1	3.4±0.5	2.8±0.6	1±0*
Rearings	51.9±4.4	53.2±4.3	27±5.1*	1±0.6*
Total SAP	25.1±3.4	20.3±2.2	21.1±2.7	3.5±0.96*
Total immobility (s)	2.21±1.1	2.63±0.8	12.9±3.2*	174±21.5*
Total LX	150±12	152±8.6	109±13*	8.4±3.29*
%wLX	15.2±3	13.4±2.3	21.4±3.9	87.7±8.65*
%w time	19.7±2.9	18.1±2.7	24.9±3.3	91.8±7.26*
Rate *W* LX	0.38±0.1	0.37±0	0.3±0.1	0.02±0.01*
Rate *B* LX	0.53±0	0.54±0	0.39±0.1*	0.12±0*
Ratio *W* immobility	0±0	0.02±0	0.06±0	0.61±0.06*
Ratio *B* immobility	0.01±0	0.01±0	0.03±0	0.06±0.03

Data are mean values±SEM. See text for further details.
* $P \leq 0.05$ versus corresponding vehicle control.

immobility and rate of wLX [$F_{2,105} \geq 5.9$, $P \leq 0.005$] and significant strain × dose interactions for total LX and total SAP [$F_{6,105} \geq 4.7$, $P \leq 0.005$]. Post-hoc tests comparing vehicle-treated animals of each strain showed that strains differed significantly on total LX (NMRI < DBA = C57) and total SAP (NMRI = DBA > C57). In NMRI mice, ZOL significantly reduced total LX at 3 mg/kg ($P < 0.001$) whereas this measure was reduced by both 1 and 3 mg/kg in C57 and DBA mice ($P < 0.01$). In NMRI mice, all doses of ZOL significantly decreased total SAP ($P \leq 0.01$), whereas this measure was decreased in DBA mice only by the 3 mg/kg dose ($P < 0.001$) and not at all in C57 mice.

NMRI. Table 7 (upper). One-way ANOVA revealed that ZOL significantly affected many other test measures in this strain, including transitions, total rearing, total immobility, % wLX, %w time and the rate of white LX [$F_{3,39} \geq 6.7$, $P \leq 0.005$; $H(3) = 13.0$, $P < 0.01$]. Post-hoc tests confirmed that the highest dose tested (3 mg/kg) significantly reduced transitions, total rearing and rate of LX in the white compartment ($P \leq 0.001$), while increasing total immobility and %w time ($P \leq 0.05$). Although not significant, an increase of ratio immobility time in both compartments was also observed at the highest dose. In view of this general pattern of behavioural suppression, the increase in %w time at 3 mg/kg must be considered a false positive arising from drug-induced sedation. However, the reduction in total SAP seen at lower doses (0.3–1 mg/kg) cannot be explained in this way, and suggests a rather selective effect of ZOL on risk assessment in this strain.

C57. Table 7 (centre). ZOL significantly altered transitions, total rearing, total immobility, rate of LX in both compartments, and ratio immobility time in both compartments [$F_{3,39} \geq 10.6$, $P \leq 0.001$; $H(3) \geq 20$, $P \leq 0.001$]. Post-hoc tests showed that the compound significantly decreased transitions (1–3 mg/kg) and total rearings (3 mg/kg) while concurrently increasing total immobility (1–3 mg/kg) ($P \leq 0.05$). These findings, together with the results of the two-way ANOVA above, clearly support a sedative effect of ZOL (1–3 mg/kg) in this strain. The novel rate and ratio measures confirmed this profile, in that ZOL decreased the rate of LX (1–3 mg/kg) in both compartments, whereas 1–3 mg/kg significantly increased ratio immobility time to a similar extent in both compartments ($P \leq 0.05$).

DBA. Table 7 (lower). Nearly all test measures were significantly influenced by ZOL in DBA mice [$F_{3,39} \geq 11.2$, $P \leq 0.001$; $H(3) \geq 16.1$, $P \leq 0.001$], the only exception being ratio immobility time in the black compartment. ZOL (1–3 mg/kg) reduced the rate of LX in the black compartment and total rearing while concurrently increasing total immobility ($P < 0.05$). The highest dose additionally reduced transitions and the rate LX in the white compartment, while increasing %wLX, %w time and ratio immobility time in the white compartment ($P \leq 0.05$). Together with the two-way ANOVA results, these data are consistent with a strong sedative effect of 1–3 mg/kg ZOL in DBA mice.

3.4. Vogel conflict (VCT)

3.4.1. CDP

Data are summarised in Fig. 4 (panel A). Two-way ANOVA on punished licking data revealed significant main effects of strain [$F_{2,186} = 65.7$, $P < 0.001$] and dose [$F_{3,186} = 5.2$, $P = 0.002$] but no interaction [$F_{6,186} = 1.5$, NS]. For latency to the 20th lick (data not shown), only a significant effect of strain was found [$F_{2,186} = 9.6$, $P < 0.001$]. In NMRI mice, CDP (5–20 mg/kg) was without significant effect on either punished licking or latencies [$F_{3,44} \leq 1.6$, NS]. By contrast, in C57 mice, CDP (10 and 20 mg/kg) produced a dose-dependent anti-conflict effect, significantly increasing punished licking ($F_{3,102} = 6.0$, $P < 0.001$) without having any effects on the latency measure [$F_{3,102} = 1.8$, NS]. In DBA mice, CDP (10 mg/kg) modestly though significantly increased punished licking [$F_{3,38} = 4.2$, $P < 0.02$), again without significantly altering latency scores [$F_{3,38} = 0.7$, NS].

3.4.2. L-838,417

Results are summarised in Fig. 4 (panel B). Due to the variable dose ranges tested in the 3 strains, the effects of L-

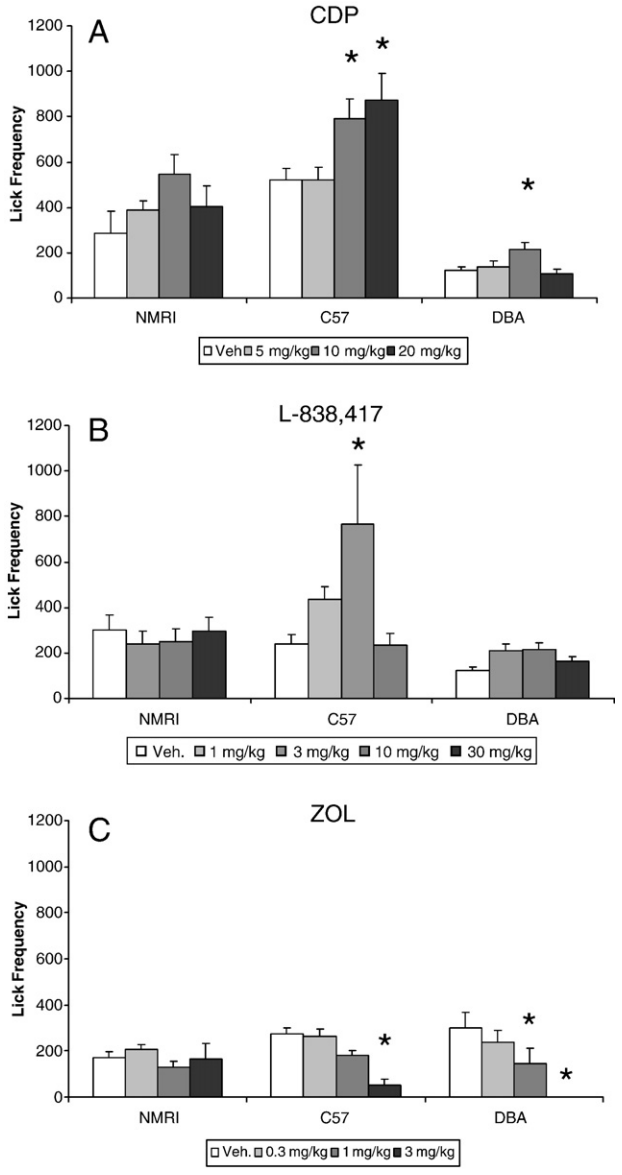

Fig. 4. The effects of CDP (5–20 mg/kg; panel A), L-838,417 (3–30 mg/kg; panel B) and ZOL (0.3–3.0 mg/kg; panel C) on lick frequency in NMRI, C57 and DBA mice exposed to the Vogel Conflict Test (VCT). Data are mean values ± SEM. See text for further details. *$P \leq$ 0.05 versus corresponding vehicle control.

838,417 on punished drinking and latency to complete 20 licks (data not shown) were analysed by one-way ANOVA on each strain individually. In NMRI mice, no overall effect of L-838,417 was obtained for either punished licking or latency to complete 20 licks [$F_{3,39} \leq 1.18$, NS]. By contrast, in C57 mice, an overall effect on punished licking was found [$F_{3,33}=4.43$, $P=0.01$] in the absence of any effect on latency to 20th lick [$F_{3,33}=0.83$, NS]. Subsequent post-hoc tests showed that 3 mg/kg significantly increased punished licking ($P<0.05$) in this strain. Although L-838,417 had no effect on punished licking in DBA mice [$F_{3,38}=2.41$, NS], it did significantly alter latency to the 20th lick [$F_{3,38}=3.19$, $P<0.05$]. Post-hoc testing revealed that 10 mg/kg significantly decreased this measure ($P<0.05$).

3.4.3. ZOL

Data are summarised in Fig. 4 (panel C). Two-way ANOVA on punished licking data failed to reveal a significant main effect for genetic strain [$F_{2,141}=2.4$, NS], although there was a significant strain × dose interaction [$F_{2,141}=4.1$, $P<0.001$]. ZOL had no effect on punished licking in NMRI mice, it significantly reduced licking in C57 (3 mg/kg) and DBA mice (1–3 mg/kg). Latency data (not shown) failed to meet two-way ANOVA criteria and were analysed with a one-way ANOVA for each strain individually. Although a significant overall effect of ZOL was found on latencies in NMRI mice [$F_{3,37}=3.3$, $P<0.05$], post-hoc tests found no significant differences between drug-treated animals and vehicle controls. However, latency measures were significantly affected by ZOL in both C57 [$F_{3,37}=27.9$, $P<0.001$] and DBA [$H(3)=19.2$, $P<0.001$] mice. Post-hoc analyses showed that latency to the 20th lick was significantly increased by ZOL in C57 (1–3 mg/kg) and DBA (3 mg/kg) mice ($P \leq 0.05$). Thus, ZOL clearly had dose-dependent sedative effects in C57 and DBA mice, with a similar (though non-significant) trend in the NMRI strain.

4. Discussion

Present results not only confirm strain differences in anxiety-like behaviour in mice but also reveal substantial variation in the 'rank order' of these strains across different behavioural models. DBA mice consistently displayed a substantially higher level of anxiety-like behaviour in the EPM and LDE when compared to the C57 and NMRI strains. The C57 strain, in particular, was characterised by a non-anxious profile in the EPM and LDE, with an approximate 50:50 distribution of time spent in the aversive:non-aversive areas of these novel environments. These findings are consistent with previous exploration-based studies that have characterised DBA mice as an relatively anxious strain (Mathis et al., 1994; Rogers et al., 1999; Kopp et al., 1999; Griebel et al., 2000; Tarantino et al., 2000; Tang et al., 2002; Carola et al., 2002) and C57 mice as a relatively non-anxious strain (Robertson, 1979; Crawley and Davis, 1982; Mathis et al., 1994; Belzung et al., 2000; Hode et al., 2000; Lepicard et al., 2000; Anisman et al., 2001; Yilmazer-Hanke et al., 2003). Despite this apparent consistency in the C57 phenotype, our data reveal that this strain was actually the most anxious strain in two other tests of anxiety-like behaviour, the ZM and VCT. Since other research groups have also found C57 mice emotionally quite reactive under certain circumstances (Crawley and Davis, 1982; Trullas and Skolnick, 1993; Parmigiani et al., 1999; van Gaalen and Steckler, 2000; Griebel et al., 2000; Avgustinovich et al., 2000; Cook et al., 2001), it would seem unwise to stereotype this (or indeed any?) mouse strain as 'anxious' or 'non-anxious' without reference to contextual details (i.e. specific test parameters and/or comparator strains).

The ZM is essentially an EPM without a central platform and was considered by its inventors to have improved pharmacological sensitivity (Shepherd et al., 1994). It was therefore quite surprising to find marked differences in levels of anxiety-like behaviour shown by the same mouse strains in these two procedures. Thus, whereas DBA mice spent similar amounts of time

in the open (20%) on both mazes, NMRI spent 40% time in the open in the EPM but only 20% time in the open on the ZM while C57 mice spent 55% time in the open on the EPM but only 10% time in the open on the ZM. This test-dependent pattern of basal anxiety suggests that the superficially similar EPM and ZM tests may actually be tapping different facets of emotionality and, together with the markedly different strain rank order in LDE versus ZM and VCT, further supports the proposal that different rodent models of anxiety-like behaviour reflect different psychological states (File, 1992; Belzung and Le Pape, 1994; Beuzen and Belzung, 1995; Rodgers, 1997; Griebel et al., 2000; Yilmazer-Hanke et al., 2003). In this context, it has been argued that, due to such differing motivations, tests such as the rat EPM and VCT involve different neurobiological substrates and that it is this distinction that is responsible for variation in pharmacological response to BZ site ligands with different agonist or subtype selectivities (e.g. Nazar et al., 1997). Despite the intuitive appeal of this argument, current pharmacological results suggest otherwise. In other words, for some mouse strains (e.g. C57) at least, the proposed distinction between exploratory- (EPM, ZM and LDE) and shock-based (VCT) tests cannot readily account for the test-dependent pharmacological profile.

To begin, our data clearly show that sensitivity to the anxiolytic effects of CDP is highly strain- and test-dependent. Consistent with previous work on various outbred strains e.g. (Crawley and Davis, 1982; Wilks et al., 1987; Mathis et al., 1994; Garrett et al., 1998; Gard et al., 2001; Choleris et al., 2001; Rodgers et al., 2002; Olivier et al., 2002) CDP exerted dose-dependent anxiolytic effects in NMRI mice in all 3 exploratory tests (EPM>ZM> LDE). The relatively low basal anxiety level displayed by NMRI mice in the EPM and LDE might have been expected to reduce the chances of detecting an anxiolytic drug effect (i.e. possible 'floor effect'). Strikingly, however, CDP significantly increased the percentage of open entries and open arm time on the EPM to 70–80% and increased percentage of time spent in the light compartment of the LDE apparatus to 80%. This phenomenon of BZ-induced 'preference' for purportedly aversive areas has previously been found in both inbred and outbred strains (Griebel et al., 2000; Belzung, 2001) but has yet to be adequately explained. Although it is difficult to appreciate what this phenomenon might mean in terms of subjectively-experienced anxiety, it may reflect increased risk-taking analogous to the effects of BZs and alcohol in clinically non-anxious humans (Lane et al., 2004, 2005). However, perhaps the most interesting aspect of CDP's profile in NMRI mice is that it was without effect in the VCT. Although the literature on shock-based conflict tasks in mice is sparse, several reports have described anxiolytic effects of CDP or diazepam in various outbred strains in such tests (Martin et al., 1993; Umezu, 1999; Liao et al., 2003; Witkin et al., 2004), we are therefore at a loss to explain the apparent resistance of NMRI mice to the anti-conflict effects of CDP in the current study.

As already noted, C57 mice displayed low basal levels of anxiety-like behaviour in the EPM and LDE, but high basal anxiety levels in the ZM and VCT. Nevertheless, these animals presented a CDP profile essentially opposite to that seen in NMRI mice, i.e. they were resistant to the anxiolytic effects of the drug in the EPM, LDE and ZM but showed a profound anti-conflict response in the VCT. This divergent profile is consistent with previous research indicating that BZ effects in C57 mice are highly test-dependent (Crawley and Davis, 1982; Mathis et al., 1994; Crawley et al., 1997; Kopp et al., 1999; Griebel et al., 2000; Lepicard et al., 2000; Gard et al., 2001; Rodgers et al., 2002), and suggests that a shock-based conflict test rather than an unconditioned exploratory test is necessary to detect anti-conflict effects of CDP in C57 mice. In the DBA strain, a third CDP pattern was recorded i.e. an anxiolytic-like response to CDP in the EPM, LDE and VCT but, curiously, no effect in the ZM. Interestingly, the magnitude of effect in the EPM and LDE was smaller compared to that observed in NMRI mice, confirming the view (Griebel et al., 2000; Belzung, 2001) that a high basal level of anxiety-like behaviour is not necessarily correlated with a high BZ sensitivity. Although anxiolytic effects of BZs (including CDP and DZ) have previously been reported in this strain (Cole and Rodgers, 1993, 1995; Griebel et al., 2000; Dalvi and Rodgers, 2001; but see Crawley et al., 1997), DBA mice have not traditionally been used in shock-based anxiety models (such as the VCT), thereby precluding comparison with previously published literature.

Unsurprisingly, sensitivity to L-838,417 also varied as a function of genetic strain and behavioural model. In NMRI mice, this novel $\alpha_{2,3,5}$-selective $GABA_A$ receptor partial agonist (McKernan et al., 2000) induced a consistent anxiolytic-like effect in the ZM, but only a marginal response in the EPM, non-specific behavioural actions in the LDE, and no effect at all in the VCT. This profile suggests that maze tests, and especially the ZM, may be particularly suited to detecting anxiolytic-like activity of L-838,417 in NMRI mice. In marked contrast, C57 mice displayed anxiolytic sensitivity to L-838,417 in the VCT and the LDE but not in either of the maze tests. It would therefore appear that the anxiolytic effect of L-838,417 in this strain is independent both on the type of model (unconditioned, non-shock-based, exploratory LDE versus conditioned, shock-based, conflict VCT) and the basal level of anxiety-like behaviour (low in LDE versus high in VCT). It is intriguing that, whereas only one test (VCT) was able to detect the anxiolytic effects of CDP (non-selective) in C57 mice, the anxiolytic sensitivity of this strain extended to include the LDE when L-838,417 (subtype-selective) was used. This phenotypic pattern suggests that the VCT and LDE may be particularly suited to detecting anxiolytic-like activity of L-838,417 in C57 mice. Despite generally higher anxiety baselines, DBA mice failed to show an anxiolytic response to L-838,417 in any of the tests, thereby strongly supporting arguments that basal anxiety per se cannot account for variable patterns of pharmacological sensitivity (Griebel et al., 2000; Belzung, 2001).

Zolpidem binds selectively to α_1 subunit-containing $GABA_A$ receptors (Pritchett and Seeburg, 1990), and the $GABA_A$-α_1 subtype has recently been shown to mediate the sedative effects of BZs (Rudolph et al., 1999; Crestani et al., 2000; McKernan et al., 2000). Our results clearly show that ZOL induced sedation (defined as a substantial reduction in behavioural output) in all strains and models. However, the degree of sedation was strain-dependent, both in terms of minimal effective dose and the number of parameters affected. More specifically, ZOL induced

sedation at a lower dose and on greater range of parameters in the DBA strain compared to NMRI and C57 strains. In this context, previous studies have noted a strain-dependent sensitivity to the sedative effects of various BZ site ligands including MDZ, ZOL, CDP and DZ (Homanics et al., 1999; Griebel et al., 2000; Rodgers et al., 2002). Together, these findings are indicative of strain variation in the CNS distribution/function of $GABA_A$-α_1 receptors.

The specific behavioural profiles of CDP and L-838,417 in the same strain and behavioural test may provide some insight into the relative importance of $GABA_A$ receptor subtypes in anxiolysis. For example, the anxiolytic effect of CDP, but not L-838,417, in NMRI and DBA mice in the EPM and LDE suggests that anxiolytic activity in these strains in these tests may require either efficacy at $GABA_A$-α_1 receptors or full agonist activity at $GABA_A$-$\alpha_{2/3}$ receptors. However, a rather different conclusion emerges from the ZM where NMRI mice were sensitive to the anxiolytic effects of both CDP and L-838,417. Although this result suggests a lack of α_1 contribution to anxiolysis in this strain and test, it should be noted that the maximum response to CDP was somewhat greater than the maximum response to L-838,417. This comparison may indicate that α_1-mediated efficacy adds to the anxiolytic-like effect or that a greater efficacy of CDP at α_2 and α_3 $GABA_A$ receptors engenders a greater anxiolytic response. Despite a similar pharmacological profile to NMRI mice in the EPM and LDE, DBA mice displayed very different responses in the ZM and VCT, i.e. CDP and L-838,417 were effective in the VCT but not ZM. Finally, in C57 mice, CDP induced anxiolytic-like effects only in the VCT while L-838,417 was anxiolytic in both the LDE and VCT. This suggests that, in the LDE, either the α_1 efficacy (i.e. sedation) of CDP counteracts the anxiolytic-like effects of this compound or, less likely, only partial agonism at the $GABA_A$-$\alpha_{2/3/5}$ subtype receptors will engender anxiolysis in this test.

Although a number of explanations have been proposed for strain differences in BZ sensitivity, Griebel et al. (2000) and Belzung (2001) have convincingly argued against the importance of basal anxiety levels while others have equally convincingly argued against variable pharmacokinetics (Belzung and Dubreuil, 1998; Weizman et al., 1999; Crabbe et al., 1998; Garrett et al., 1998). In this context, early binding studies using the 'anxious' BALB/c and 'non-anxious' C57 strains revealed the former to display significantly lower total brain BZ receptor density/affinity compared with the latter (Robertson, 1979; Chapouthier et al., 1991; Mihic et al., 1992), a difference subsequently refined to the amygdaloid complex (Hode et al., 2000). Unfortunately, this emerging mechanistic interpretation has since been undermined by Yilmazer-Hanke et al. (2003) who report BALB/c mice as having a higher density of $GABA_A$ receptors in the amygdala compared to C57 mice. Although these authors also failed to find any significant correlations between amygdaloid $GABA_A$ receptor density and anxiety level in EPM or fear-sensitized acoustic startle, this aspect of their work is at least partly compromised by the absence of the normal BALB/c versus C57 strain difference in basal anxiety levels. Despite these disappointing results, it is worth noting that NMRI and DBA strains were also included in this study and that the strain rank order of basal anxiety (both tests) was identical to that seen in the EPM and LDE tests in the present research i.e. DBA > C57 = NMRI. Since the strain rank order of amygdaloid $GABA_A$ receptor density was reported to be DBA < C57 = NMRI, high basal anxiety in these tests may be inversely linked to low amygdaloid $GABA_A$ receptor density, a relationship not inconsistent with the early binding work (Robertson, 1979; Chapouthier et al., 1991; Mihic et al., 1992; Hode et al., 2000).

To our knowledge, only one study to date has explored possible strain differences in the neuroanatomical distribution of various BZ-binding $GABA_A$ subunits (DuBois et al., 2006). This work contrasted the behavioural profiles of DBA and C57 mice in the LDE and open field tests and, consistent with present results, found DBA mice to display the higher levels of basal anxiety. The results also demonstrated that basolateral amygdaloid (BLA) neurons from DBA mice were characterised by greater GABA-gated responses and higher expression of α_2 subunit mRNA than those from C57 mice, leading the authors to suggest a causal relationship with the strain difference in basal anxiety. At first glance, the reports by Yilmazer-Hanke et al. (2003) and Dubois et al. (2006) appear contradictory in terms of the relationship between basal anxiety level and amygdaloid $GABA_A$ receptor expression. However, whereas the former measured the general expression of $GABA_A$ receptors in the amygdala (i.e. including all constituent α subtypes), the latter focused exclusively on α_2 subtype-containing GABAergic neurons. Overall, therefore, these findings suggest that the currently observed strain difference in basal levels of anxiety and pharmacological response may be intimately related to the differential expression and/or function of certain $GABA_A$ receptor subunits in key structures such as the amygdala.

As current findings derive from a large number of experiments conducted across two locations, a final caveat may be in order. Of particular relevance is an already classic paper by Crabbe et al. (1999) reporting the results of a multi-centre study designed to phenotype a large number of mouse strains in a wide range of behavioural tasks (including several of those used in the current investigation). Despite major efforts to standardise methodology, substantial variation in results (including strain rank order in tests of anxiety-like behaviour) was often observed across the participating laboratories. These findings imply that, even when all basic procedures are rigorously standardised, the 'environment of testing' can still have a significant bearing on the outcome of behavioural experiments. Ideally, the current work should have been conducted in a single laboratory with animals from the same source held under uniform conditions and subjected to identical husbandry and testing routines. In the present study, and largely for reasons of local expertise, EPM and LDE experiments were run in Leeds while ZM and VCT studies were run in Copenhagen. Not only this but, for practical reasons, animals for the UK and Danish studies were obtained from different suppliers (although we did try where possible to minimise this potential confound by using Harlan as primary source) and there were some other site variations (e.g. acclimatisation periods and cleaning regimens) over which we had negligible control. Although it is conceivable that such

methodological variation represents a significant confound, we must emphasise that a (if not the) major 'environmental' variable (the experimenter) was held constant across the studies while, irrespective of location, each experiment had its own controls and was scored blind to treatment condition. Furthermore, as our results did not show consistent behavioural and/or pharmacological differences as a function of test location (i.e. patterns did not consistently group as EPM and LDE versus ZM and VCT), we have every confidence in the reliability and validity of our major conclusions.

In summary, current findings demonstrate that different mouse strains and different models of anxiety-like behaviour have differential sensitivity to compounds that vary in $GABA_A$ subtype selectivity. More specifically, our database implies that it may not actually be possible to identify a single mouse strain or a single behavioural model that is consistently sensitive to non-selective BZ ligands such as CDP and subtype-selective compounds such as L-838,417. To complicate matters even further, our results show that a positive response to CDP in a given test and strain does not necessarily predict a positive response to L-838,417 in the same test and strain. It would therefore seem prudent to in future employ combinations of the most appropriate tests and genotypes i.e. the term 'animal model of anxiety' should be extended to incorporate both the behavioural test and a specific genotype (Rodgers, 2006), e.g. detection of L-838,417 anxiolysis through the use of NMRI mice in the ZM (but not EPM, LDE or VCT) or C57 mice in VCT and LDE (but not EPM or ZM). Further studies, using additional subtype-selective tools such as TPA023, TP003, SL651498 in these mouse strains and anxiety tests, should provide more insight into whether it is possible to define test systems (i.e. mouse strain and model combinations) for drugs with specific selectivity profiles. Although this may seem a daunting task given the wide range of available strains and behavioural models, the judicious choice of more than one strain and one model should be sufficient to provide some very relevant pointers — a conclusion that is probably valid not only for the $GABA_A$ receptor system but also all other anxiety-relevant neurobiological systems.

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