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Effects of Diazepam and Buspirone on the Behaviour of Wild Voles (*Microtus socialis*) in Two Models of Anxiety

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HENDRIE, C. A., D. EILAM AND S. M. WEISS. *Effects of diazepam and busipirone on the behaviour of wild voles (Microtus socialis) in two models of anxiety.* PHARMACOL BIOCHEM BEHAV **58**(2) 573–576, 1997.—Exploration models of anxiety rely almost universally on the use of laboratory species. Furthermore, the spontaneous patterns of locomotion displayed are often interpreted as being an expression of antipredator defense. However, there is no direct link between the experience of these animals and the proposed motivation for their behaviour. To address this problem, the behaviour of wild trapped voles (*Microtus socialis*), a small-rodent species that is heavily predated upon, was examined in the elevated plus-maze and the black/white exploration model. It was hypothesised that the patterns of locomotion in these exploration models of anxiety should be similar to those reported for laboratory animals if the reactions of the laboratory animals are related to antipredator defense. Data revealed that voles show a similar preference for the protected areas in these models (closed arms or dark section) and that this preference can be modified by buspirone and diazepam. Interestingly, although the effective doses of each drug was the same within each model, it differed between models, with the minimum effective doses of these compounds being lower in the black/white exploration model (1 mg/kg) than in the elevated plus-maze (4 mg/kg). These data provide valuable information concerning the actions of anxiolytic compounds in wild trapped animals as assessed by formal laboratory models and provide useful verification that findings in these models may be generalised to species other than laboratory rodents. © 1997 Elsevier Science Inc.

Microtus Voles Wild animals Buspirone Diazepam Elevated plus-maze Black/white exploration model Anxiety Anxiolytics

OVER the years, a large array of tests designed to identify anxiolytic drugs have been developed, which may be divided on the basis of the observation of conditioned or unconditioned responses [for review, see (5)]. Conditioned responses are based on fear induced by secondary stimuli (aversive conditioning), and unconditioned responses assume that the responses seen are induced by innate or spontaneous fear (8). In contrast to "conditioning" models, tests employing unconditioned responses, by their very nature, often produce data that are equivocal as behaviour in these models may be modulated by alterations in locomotor activity or fear (e.g., (9)]. As such, a change in locomotor patterning may be due equally to an increase or decrease in anxiety levels, a simple effect on locomotor behaviour per se or both.

In addition to these interpretational problems, the source of the proposed spontaneous fear, which proportedly motivates behaviour in these models, remains unidentified [e.g., (13)]. Although it is implicit to the interpretation of drug effects, it is rarely explicitly stated that aspects of the test apparatus are assumed to offer protection from potential predators (4). That is, changes in locomotor patterning from a preference for "protected" (e.g., dark or closed) areas to equal amounts of behaviour in protected and unprotected areas are viewed as being due to changes in the animal's cognitive appraisal or "risk assessment" of the likelihood of potential predatory attack [e.g., (12)]. Although this interpretation has certain intuitive appeal, the behaviours expressed by laboratory animals in these circumstances are a consequence of se-

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lection pressures exerted on the ancestral wild stock. As such, it is clear, regardless of the explanations forwarded, that there is no direct link between the experience of the laboratory animal and the proposed motivation for its behaviour. Therefore, it is important to determine whether similar behavioural profiles are seen in wild trapped animals that have been unequivocally exposed to the possibility of predation in their natural environment to lend weight to popular interpretations of behaviour in exploration models of anxiety.

Microtus socialis are 35–50-g, 10-cm long (excluding tail), burrow-dwelling rodents which eat seeds and green vegetation. Their eyes and external ears are small, and their limbs and tail are short (2–3 cm). They have a high fecundity, are heavily preyed upon and show specific and appropriate defensive reactions when exposed to partial predator stimuli, such as an owl call (14). Therefore, this species was selected as being appropriate for use in the present studies. To further determine generalisability, two models of anxiety, which rely on a change in preference from either dark [black/white exploration model (3)] or closed [elevated plus-maze (11)] areas, were also employed. A benzodiazepine (BZP) anxiolytic and non-BZP antianxiety agent were selected as reference drugs in view of the wealth of data relating to their effects in the clinic and in the laboratory [for review, see (12)].

In these studies, three hypotheses were under examination: (a) wild animals would show the same preference for protected areas as those reported for their laboratory counterparts; (b) there may be differences in patterns of behaviour seen in the black/white exploration model and the elevated plus-maze; and (c) there may be differences in the effects of diazepam and buspirone under these conditions.

METHODS

Animals and Housing

Male and female *Microtus socialis* were obtained by live trapping from the Lod Region of Israel, near Ben Gurion Airport. Animals were then transported to the Research Zoo, University of Tel-Aviv, and held in mixed-sex groups of 6–10 in steel walled cages (70 × 40 × 20 cm) under natural lighting conditions for at least 2 months prior to testing. Food and fluid, in the form of seeds, diced carrots, lettuce and apples, were provided once a day.

All studies were conducted under the regulations of the Israeli Nature Reserve Authority and the Ethics Committee for animal experimentation in the University of Tel-Aviv. In addition, C. A. H. holds a Personal Licence (PIL 50/01058) to conduct these procedures within the scope of the UK Animals: Scientific Procedures Act (1986).

Apparatus

Two behavioural models were used: (a) the blackwhite exploration model, an open-topped box (45 × 27 × 27 cm high) that is painted one-third black and is illuminated under dim red light (1 × 60 W). The remaining two-thirds is painted white and is brightly illuminated (2 × 60 W). A partition with a small opening (7.5 × 7.5 cm) divided the two areas and the floor area is lined with 9-cm squares; (b) the elevated plus-maze, which is comprised of two "open" (30 × 5 × 0.25 cm) and two "closed" (30 × 5 × 15 cm) arms arranged in the shape of a plus sign around a 5 × 5-cm central square. A single support raised the apparatus 45 cm from floor level.

In the black/white exploration model, animals show a natural preference for the black, dimly illuminated section. Anxiolysis is indicated when activity in both sections is statistically equal. In the plus-maze, anxiolysis is similarly indicated when the animal's natural tendency to remain mostly in the closed arms is reduced and their tendency to explore the open arms is increased such that they are also statistically equal.

Drugs

Diazepam and buspirone (Sigma Chemical Company Ltd., UK) were suspended or dissolved in 0.9% saline, which alone served as a vehicle control. Drugs were administered intraperitoneally in a volume of 10 ml/kg.

Procedure

All testing was conducted under dim red light (2 × 60 W) starting at dusk and ending 3 h later (1700–2000). This time was chosen to coincide with the animal's normal active period at the beginning of the dark phase and restricted as the animals began their sleep cycle after this. Voles were allocated in randomised order, counterbalanced for sex and drug, to receive saline vehicle or one of the doses of buspirone or diazepam (0–4 mg/kg) and to be placed in either the black/white exploration model or the elevated plus-maze (n's = 7 and 9). Thirty minutes postinjection, animals were placed into their allocated test apparatus, with their behaviour being recorded on videotape over the next 5 min for subsequent analysis. The test apparatus was then cloth wiped between subjects and fecal boli removed before the next animal was introduced. Each animal was used once only. Each animal was used once. The experimenters remained blind to drug treatment throughout, and codes were broken only after the behavioural analysis was complete.

Statistics

Data were analysed using two-factor analysis of variance (ANOVA) with repeated measures on the last factor to account for behaviour in each section of the apparatus used. Follow-up tests were performed by using the appropriate error term from the ANOVA table.

RESULTS

Black/white Exploration Model

Two-factor ANOVA (repeated measures on last factor) revealed significant effects of diazepam on line crossings [$F(1,26) = 5.53$, $p < 0.05$] and a significant drug × area interaction with regard to rearing [$F(3,26) = 4.92$, $p < 0.001$] (Fig. 1). In the case of line crossings, this was found to be due to significant differences between activity in the black and white areas in saline treated animals only ($t_d(26) = 3.68$, $p < 0.01$) as was the case with rearing ($t_d = 2.63$, $p < 0.05$) indicating an anxiolytic action of all doses of diazepam employed. ANOVA also revleated there to be effects of buspirone as indicated by a significant drug × area interaction ($F(3,26) = 8.09$, $p < 0.0001$) on line crossing and rearing ($F(3,26) = 6.01$, $p < 0.001$). Dunnett's t test indicated that the effects on line crossing were due to significant differences in saline-treated animals only [$t_d(26) = 3.32$, $p < 0.01$]. The effects on rearing were due to a similar pattern of results [$t_d(26) = 3.09$, $p < 0.01$]. These data indicate anxiolytic action induced by all employed doses of buspirone. Examination of total activity to determine possible sedative action revealed that, whereas diazepam was without effect, 1 mg/kg buspirone significantly reduced rearing [$F(1,26) = 4.34$, $p < 0.05$] and 4 mg/kg ap-

proached significance [$F(1,26) = 4.06$, p = 0.054]. These data suggest that the "anxiolytic" effects produced by these doses may be secondary to their effects on motor activity.

Elevated Plus-Maze

Two-factor ANOVA (repeated measures on the last factor) revealed significant closed/open arm effects [$F(1,23) = 14.3$, $p < 0.0001$] in buspirone-treated animals (Fig. 2). Follow-up tests revealed these effects to be due to significant differences in time spent in each arm in voles treated with saline [$t_d(23) = 3.89$, $p < 0.001$] and 2 mg/kg buspirone [$t_d(23) = 5.1$, $p < 0.001$]. There were no significant differences in open/closed arm activity in animals treated with 4 mg/kg buspirone [$t_d(23) = 0.36$, NS]. With regard to diazepam, a similar pattern emerged, with significant main effects for open/closed arm activity [$F(1,23) = 8.6$, $p < 0.001$], which was due to significant differences in open/closed arm activity in animals treated with saline [$t_d(23) = 3.57$, $p < 0.001$] and 2 mg/kg diazepam [$t_d(23) = 2.66$, $p < 0.05$]. Four milligrams per kilogram of diazepam reduced these differences to nonsignificance [$t_d(23) = 1.05$, NS], suggesting that this dose is anxiolytic.

Examination of total arm entries to determine possible sedative action revealed that neither buspirone [$F(2,23) = 0.31$, NS] nor diazepam [$F(2,23) = 0.14$, NS] had any effect on this measure, which in the case of buspirone contrasts with the suggestion of sedative action indicated by the black/white exploration model.

DISCUSSION

Current data reveal that (a) wild rodents show a preference for protected areas within the test apparatus; (b) the proportions of behaviour seen in saline-treated animals in either closed (elevated plus-maze) or dark (black/white exploration model) area were roughly equivalent (in the order of 65%); and (c) although the efficacy of diazepam and buspirone in altering the preference for the protected area, to equal expression of behaviour in each area, was approximately the same within each model, the effective dose of each drug changed across models.

In the elevated plus-maze, both compounds were active at, but not below, 4 mg/kg. In contrast, in the black/white exploration model, buspirone and diazepam were active at all doses tested, suggesting that this model is more sensitive to anxiolytic drug effects with these animals. In keeping with this suggestion of increased sensitivity to drug action in the black/white exploration model, the effects of buspirone appeared to be confounded by possible sedative action, an effect that was not seen in the elevated plus-maze. Although restrictions enforced by the availability of animals prevented the examination of 1 mg/kg buspirone in this model, direct comparisons could be made with 4 mg/kg buspirone as this dose was tested in both the black/white exploration model and elevated plus-maze. Data revealed a very strong indication of sedative action induced by buspirone in the black/white exploration model but not the plus-maze. There are several possibilities to account for these suggested differences in drug sensitivity.

Firstly, a more detailed analysis of behaviour on the plus-maze may have revealed that these drugs were effective at doses lower than those indicated by the very simple measures employed (12). However, this possibility does not account for the lack of the sedative action of buspirone that was seen in the black/white exploration model. It may also be assumed that the adoption of ethological measures in this test may have indicated effects at lower doses of buspirone and diazepam, had these been investigated. Secondly, these data may be a consequence

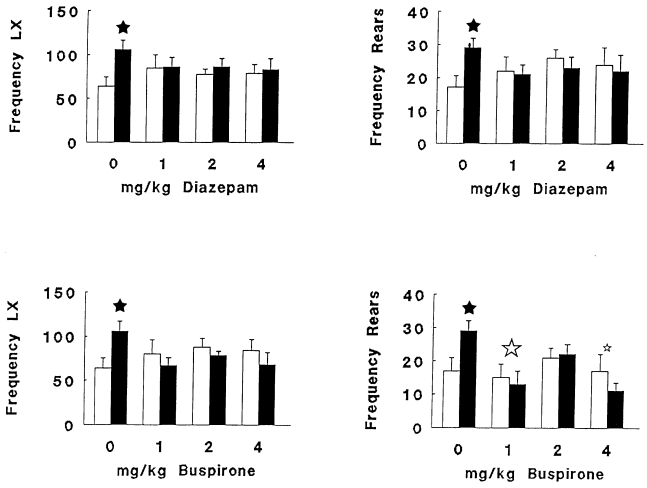

FIG. 1. The effects of anxiolytics on the behaviour of wild voles in the black/white exploration model. Data are presented as means (± SEM) of the frequency of line crossings (left) and rearing (right) in the light (open bars) or dark (solid bars) section of the apparatus. These findings indicate that all doses of diazepam or buspirone produce an anxiolytic profile, as indicated by the failure of these animals to show the preference for the black area as seen with vehicle controls. However, the effects of buspirone on rearing were confounded by a possible sedative action because the total numbers of rears was significantly reduced by 1 mg/kg, an effect that approached significance in animals treated with 4 mg/kg. Filled stars, $p < 0.05$ vs. behaviour in light area; large open stars, $p < 0.05$; small open stars, $p < 0.06$ vs. total rears of saline-treated control animals.

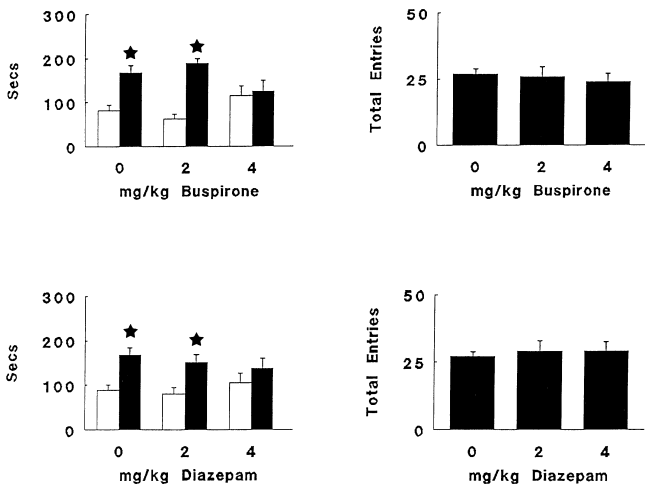

FIG. 2. The effects of anxiolytics on the behaviour of wild voles in the elevated plus-maze. Data are presented as means (± SEM) of time spent on the open (open bars) or closed (solid bars) arms of the apparatus. Findings suggest that 4 mg/kg of either diazepam or buspirone induce an anxiolyticlike profile, as indicated by the failure of these animals to exhibit the preference for the closed arms as seen with vehicle controls. Data presented on the right hand graphs indicate that these effects are independent of a confounding sedative action as total entries remained unaffected by any dose of buspirone or diazepam examined. Star, $p < 0.05$ vs. behaviour in open arms.

of the differential "closed" vs. "dark" properties of each model and strongly suggest, using current measures, that the behaviours expressed in the open/closed areas of the elevated plus-maze are more resistant to alteration by anxiolytics than those seen in the light/dark areas of black/white exploration model. Finally, and related to the second point, the two models may measure different but related phenomena. That is, it may the case that being placed on the elevated plus maze had relatively little effect on these animals' normal anxiety tone, while being placed in the black/white exploration model may have been more stressful, leading to a concatentation of normal anxiety and that induced by the environment itself. This, in turn, may have led to an increased sensitivity to drug action. Importantly, the opposite case may be argued. Nonetheless, these findings have clear parallels with clinical data indicating that anxiolytics are much more effective in the treatment of state anxiety or "fear" than major, apparently spontaneous, anxiety states related to "trait" [e.g., (7)] and may provide a method by which to differentiate them.

In this context, aspects of the present study demonstrate the need for concordance between "species-specific defensive behaviour" and the models of anxiety employed. For example, examination of the defensive behaviour of jerboa (*Jaculus jaculus*) in reaction to stimuli indicating the presence of a predator revealed their preferred response in enclosed spaces to be freezing and crouching (14). These behaviours are considered confounds in exploration models because they are indicative of sedative action. Attempts were also made to examine the behaviour of spiny mice (*Acomys dimidiatus*) on the elevated plus-maze. However, these animals demonstrated a pattern of behaviour completely opposite to that which was expected. The most likely explanation for this anomolous behaviour is that the very long vibrissae of these animals dissuaded them from entering the closed arms and enabled the detection of thygmotactic cues from the underside of the open arms. Therefore, these data demonstrate that when a variety of species are available, it is only those which display particular antipredator defense strategies that are suitable for use in investigations using exploration models such as the elevated plus-maze and the black/white exploration model. This point is illustrated further by studies showing that laboratory mouse strains also differ in their propensity to enter the closed arms of the elevated plus-maze, with the BALB/c strain demonstrating a pattern of activity similar to that seen in spiny mice (2).

In conclusion, as far as the authors are aware, current data provide the first information concerning the effects of anxiolytic drug action, as assessed in formal laboratory models, on the behaviour of wild trapped animals, although, such compounds have been assessed in "nonstandard" models (e.g. (1)) and informally (e.g. (10)). As such, these findings provide useful verification that anxiolytic drug action may be detected in nonlaboratory animals using these models and add weight to the hypothesis that behavioural patterns seen in the laboratory may be related to antipredator defense. However, to reemphasise the importance of species concordance with the test situation, it has recently been argued that the behaviour in these tests of a territorial speices, such as the mouse, may be more related to anxiety relating to intraspecific confrontation than interspecific predator–prey interactions (6). Possibly these models should be accepted at face value. They are drug screens with predictive validity only and attempts to interpret behaviour on them should be restricted to the data in hand.

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