Pharmacology Biochemistry & Behavior, Vol. 18, pp. 37–40, 1983. Printed in the U.S.A.

Further Characterization of a Simple, Automated Exploratory Model for the Anxiolytic Effects of Benzodiazepines

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Received 22 July 1982

BLUMSTEIN, L. K. AND J. N. CRAWLEY. *Further characterization of a simple, automated exploratory model for the anxiolytic effects of benzodiazepines.* PHARMACOL BIOCHEM BEHAV **18**(1) 37–40, 1983.—Anxiolytics specifically increase the number of exploratory transitions in a two-chambered model system for anxiety in mice. Characterization of parameters to optimize and standardize this model required analysis of multiple use of test animals, intertrial interval, and circadean variability. Time of day did not affect exploratory activity in mice treated with vehicle or diazepam between 10 a.m. and 11 p.m. Intertrial intervals of 1, 3, 5, or 7 days were equally effective. The diazepam-induced increase in exploratory activity was significant over the first three uses of test animals. These data recommend reuse of mice to a maximum of three trials, throughout the daytime or evening hours of their lighting schedule.

Automated exploratory model Anxiolytics Benzodiazepines

A TWO-chambered exploratory behavior model for anxiety in mice has been described and characterized for its pharmacologic specificity to anxiolytics [4, 5, 6, 7]. Basically a naturalistic modification of the conflict test, this model titrates the inherent tendency of mice to explore a novel environment against their natural avoidance of a brightly lighted open field. The testing apparatus records the numbers of transitions made by a mouse between a highly illuminated open-field compartment and a dark, enclosed compartment. Benzodiazepines significantly increase the number of exploratory transitions. Rank-order potencies, dose-response curves, and pharmacological specificity for anxiolytics are similar to those reported for other animal models of anxiety [1, 2, 3, 8, 9, 11, 12, 13, 14, 15, 16].

Proposal of a new animal behavior model system requires further characterization of the optimal test parameters for its application. Several parameters require careful analysis at this stage of model evaluation. Time of day may have a major influence on exploratory behavior and responsiveness to diazepam, especially on exploratory behavior and responsiveness to diazepam, especially in view of reported circadean rhythms in number of brain benzodiazepine binding sites [17]. Reuse of animals must be analyzed for possible habituation to the novelty of the exploratory apparatus, and for the influence of habituation to handling and intraperitoneal injections. Number of days intervening between benzodiazepine challenges may be critical, since metabolites of benzodiazepines persist in vivo for several days in man [10]. This report addresses the issues of multiple use, intertrial interval, and circadean variability, to provide a more complete analysis of test parameters relevent to benzodiazepine responsiveness in the proposed model.

METHOD

Male C57Bl/6J mice (Jackson Laboratory, Bar Harbor, Maine) 18–25 g, were housed in groups of ten in animal quarters controlled for temperature and humidity. Food and water were available ad lib. Light cycle was 6 a.m. lights on–6 p.m. lights off. Experimental testing was begun at least eight days after arrival and one day after release from site quarantine. Home cages were brought from the animal quarters to the testing laboratory one hour before testing, to control for transport stress and habituation to test room conditions.

Each animal was individually tested in a ten minute session on the exploratory apparatus previously described [4, 5, 6, 7]. In brief, a polypropylene animal cage was partitioned into a small, darkened compartment and a large highly illuminated compartment. Photocells across the partition detect transitions between the two compartments. Number of transitions are electronically tallied for the preprogrammed session length. In Experiment 1, naive mice were tested in six trials, at 2–3 day intervals over a two week period, to determine the effects of multiple use of animals. Three treatments were randomized throughout the group: diazepam 0.5 mg/kg, vehicle controls, and uninjected controls for handling stress, N=15 for each treatment. In Experiment 2, the number of days intervening between repeated testing was varied in a separate group of mice, with randomized vehicle or diazepam treatments. Each animal was used twice, e.g., day 0 and day 1, day 0 and day 3, day 0 and day 5, and day 0 and day 7, N=10 for each group. In Experiment 3, mice were tested throughout the time period 10 a.m.–11 p.m., using alternate vehicle and diazepam treat-

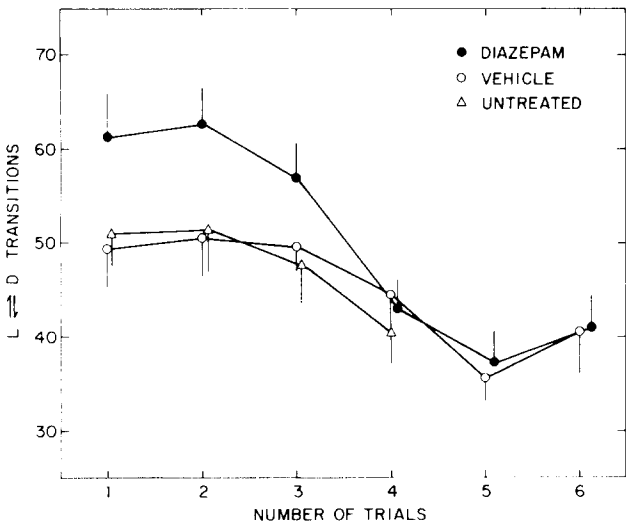

FIG. 1. C57Bl/6J male mice were repeatedly tested over a two week period in an exploratory behavior model for anxiolytics. Number of exploratory transitions between the light and dark compartments of the model system were significantly increased by diazepam 0.5 mg/kg IP on the first three trials ($p<0.01$), as compared to vehicle-treated and untreated control mice. Each point represents mean± S.E.M. of 15 mice.

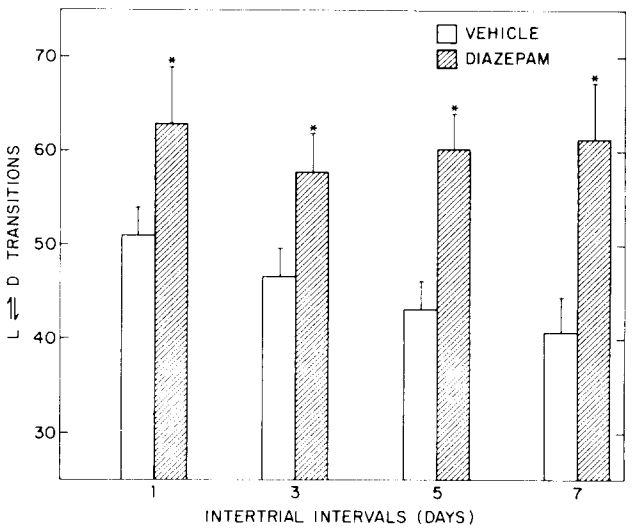

FIG. 2. Number of days between repeated testings with diazepam 0.5 mg/kg IP or vehicle. Values represent mean±S.E.M. for 10 mice. *$p<0.01$ diazepam versus vehicle. No significant differences were detected by Analysis of Variance across inter-trial intervals for vehicle treated mice or for diazepam treated mice.

ments. The sequence was replicated three times with separate groups of 5 naive mice per hour over a one month period.

Intraperitoneal injections of diazepam or vehicle were administered in a volume of 5 ml/kg, 30 minutes before testing. Diazepam (gift of Hoffman LaRoche, Nutley, NJ) was dissolved in a vehicle of 2% ethyl alcohol-2% propylene glycol-96% physiological saline. The dose of 0.5 mg/kg diazepam was chosen on the basis of highly significant increases in exploratory transitions at this dose in the C57Bl/6J strain previously described [4]. One-way Analysis of Variance was used to determine significant effects over treatment trials, with Duncan's Multiple Range Test for significance of individual means post-hoc within each treatment. The significance of the diazepam-induced increase in exploratory transitions as compared to vehicle controls was determined by one-tailed t-tests in Experiments 1 and 2.

Anecdotal observations throughout our studies indicate that environmental stresses must be controlled to avoid fluctuations in baseline exploratory behaviors. Travel during shipment, changing of cage litter, sudden loud noises, vibrations as a result of building construction, and major changes in the facility conditions, all served to increase the magnitude and variability of exploratory transitions in vehicle controls. Restriction of testing to quiet laboratory conditions, at least one hour of test-room habituation, and one week of habituation after arrival at the site were enforced to eliminate these sources of variability.

RESULTS

Experiment 1

Diazepam increased exploratory transitions above vehicle controls ($p<0.01$ as compared by t-test statistics) on the first, second, and third trials only (Fig. 1). One way analysis of variance showed a significant decline in diazepam-increased exploratory transitions over the six trials, $F(5,84)=9.3$, $p<0.01$. One way analysis of variance showed no significant differences within the vehicle treatment group, $F(5,84)=2.03$, NS, or within the untreated control group over the six trials, $F(3,56)=1.56$, NS. The untreated control group was dropped after trial 4, since it was similar to the vehicle control group, and since all three groups showed similar values by the fourth trial.

Experiment 2

Figure 2 gives values for the second test day, in which interval between the first and second tests is varied. Number of days between testing did not affect exploratory transitions in either vehicle-treated, $F(3,36)=2.66$, NS, or diazepam-treated, $F(3,36)=1.81$, NS, mice. Within each day of testing, diazepam significantly increased the number of transitions above vehicle control values ($p<0.01$ by one-tailed t-test statistics).

Experiment 3

No circadean variability in exploratory behavior was detected between 10 a.m. and 11 p.m. for either vehicle-treated, $F(13,196)=1.26$, NS, or diazepam-treated mice, $F(13,196)=1.66$, NS. As seen in Fig. 3a–b, there were no trends toward peaks or troughs in either vehicle or diazepam-treated mice throughout the daytime and evening hours. (Statistical analysis demonstrated no significant circadean effects within the vehicle treatment, or within the diazepam treatment. No comparisons between vehicle and diazepam are made or claimed in Experiment 3.)

DISCUSSION

Validation of a new animal model for anxiolytics requires a thorough description of the test parameters which yield optimal effectiveness in this model system. The results of the three experiments described in this report suggest that

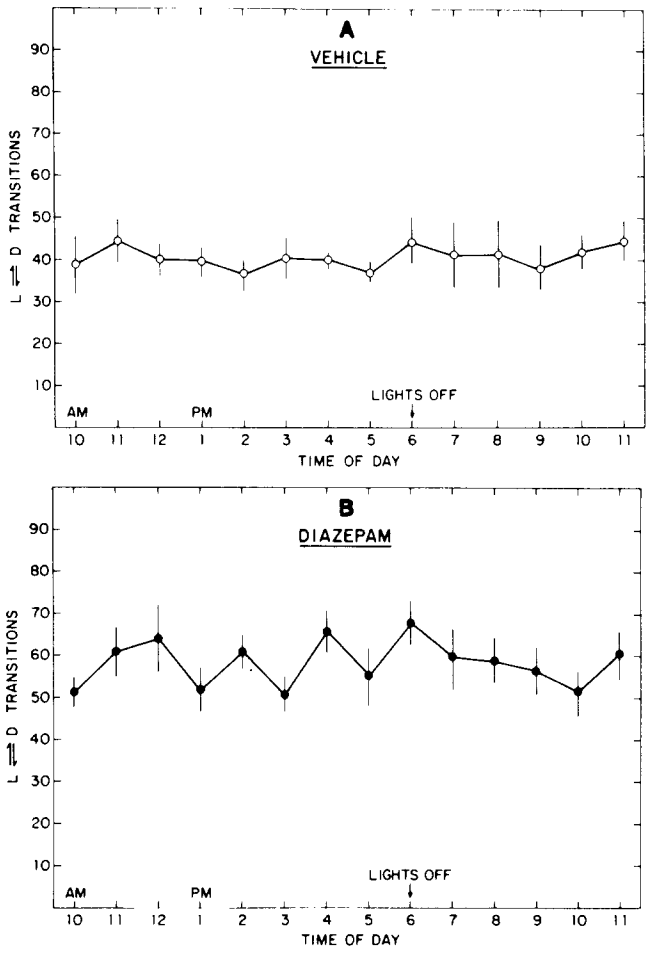

FIG. 3. Mouse exploratory behavior was tested during daytime and evening phases of the light cycle (a) vehicle controls; (b) diazepam treated, 0.5 mg/kg IP. Each data point represents mean±S.E.M. of 15 mice, N=5 in each of three replicates. Vehicle baseline activity and the diazepam-induced increase in exploratory activity remained constant over the hours tested, 10 a.m.–11 p.m.

group-housed C57Bl/6J male mice can routinely be used repeatedly up to three times. Interval between treatments and time of day do not appear to be critical in designing the test protocol.

We postulated that the proposed animal model for anxiety in mice represents a conflict between the tendencies of mice to explore a novel environment and the aversive properties of a brightly-lit open field [4]. Habituation to the novelty of a test environment would shift the balance between the conflicting tendencies, resulting in less exploration. The data of Experiment 1 illustrate a reduction in exploration by diazepam-treated mice by the fourth trial. This reduction could represent a test-chamber habituation effect as well as a drug-tolerance effect. Since the mechanism underlying the reduced responsiveness to diazepam may involve several factors, reuse of mice which habituate quickly to novel environments should be avoided.

The results of Experiment 2 suggest that mice can be reused with only one day between trials. Although benzodiazepines and their metabolites persist for many hours and days after acute administration, there is little evidence for the development of tolerance to their anxiolytic effects at the dose level used in this study [10].

The lack of circadean rhythmicity in benzodiazepine responsiveness in this model system is interesting in light of the reported circadean rhythms in number of benzodiazepine binding sites in rat brain [17]. A species difference may be involved. Another possibility is the magnitude of circadean receptor changes is relatively small as compared to an exogenous dose of 0.5 mg/kg diazepam. It is intersting to speculate that the anxiolytic effects of benzodiazepines may be mediated through specific anatomical sites, which may be constantly sensitive to environmental threats rather than subject to circadean rhythms.

Taken together with our previously reported data, these findings recommend the use of male C57Bl/6J mice, usable throughout the daytime or evening hours of their lighting schedule, for a maximum of three uses per animal. The persistence of the diazepam effect on exploratory activity across the described parameters supports the usefulness of this model system for routine analysis of pharmacological agents on anxiety-related behaviors.

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