# *The Effects Of Low Doses of Diazepem on Rat Behavior*

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## Introduction

Diazepam was among the first of the benzodiazepine (BZD) class of drugs to be used to treat anxiety. Benzodiazepines are characterized by anxiolytic, anticonvulsant, muscle relaxing, amnesic, sedation, and hypnotic effects. These effects are thought to be due to an overall "system slowing" effect caused by enhancing the inhbitory action of GABA, a neurotransmitter which is found throughout the body and brain. Benzodiazepines are fast acting. As such they have been a popular class of anxiolytics used to test anxiety behavior measures in rats (sources).

One of these anxiety measures is the elevated plus maze, a behavioral test which can be used to observe and measure rats exploratory behavior in and out of dark-enclosed spaces as well as brighter-open areas. Rats, being nocturnal, feel more comfortable in the dark-enclosed area of the elevated plus maze. Increased levels of anxiety cause rats to spend more time in the dark-enclosed arms. In our study we looked at the effect of diazepam on a number of different anxiety measures in rats given either 0.0, 0.5 or 1.0 mg/kg of diazepam using the elevated plus maze.

**Hypothesis:** There will be a dose-dependent relationship between measures of anxiety and drug dose size.

- As dose size increases, so should entries into, and time spent in open-arms of the elevated plus maze
- As dose size decreases, so should entries into and time spent in closed-arms of the elevated plus maze

## Methods

### Subjects

Twenty-six Adult male Sprague-Dawley rats were divided into three groups: a saline-injection group (n = 8), a group given an injection of 0.5 mg/kg diazepam (n = 8) and 1.0 mg/kg diazepam (n = 8). Rats were randomly paired in plastic cages.

### Apparatus

An elevated plus maze was used. The maze included 4 arms, two of which were "open-arms", which have nothing on either side, two of which were "closed-arms", which had two 41 cm tall walls on both sides of the arm. Closed and open were are present directly across from each other on the maze. Each arm was 51 cm in length. The center between arms is 10 x 10 cm . The entire maze was at a height of 76 cm off of the ground.

### Procedure

- Rats were given an injection of saline (0.9%), or diazepam (0.5 or 1.0 mg/kg) approximately 30 minutes before testing.
- Students from an upper level psychology course observed pairs of rats, one at a time.
- All lights in lab were off except for an overhead red light.
- Students were blind to which injection group rats belonged to.
- Rats were placed in elevated plus maze in the center square facing a predetermined random arm for each rat.
- Rats were observed for exactly 5 min.
- **Measures (see table 1) were calculated during observation**
- **Mazes were cleaned with soap after each test.**
- **Steps above were repeated for each pair of rats.**

| Measure | How it was calculated |
|---|---|
| Entries into open or closed-arms | All four paws enter arm |
| Time spent in open or closed-arms | Using a stopwatch |
| Rears | Front two paws off of the ground |
| Head Dips | Animal dips head below arm level |
| Slips | If rat falls, but not off platform |
| Falls | If animal fell from platform |
| Defecations | Counted after test |
| Total entries | Closed entries + open entries |

*Table 1.* A list of test measures and how they were calculated

## Results

One way ANOVAs were used to find significant effects among each measure of locomotion and anxiety between diazepam dosages. Post-hoc pairwise comparisons were done using Tukey's HSD tests for each measure.

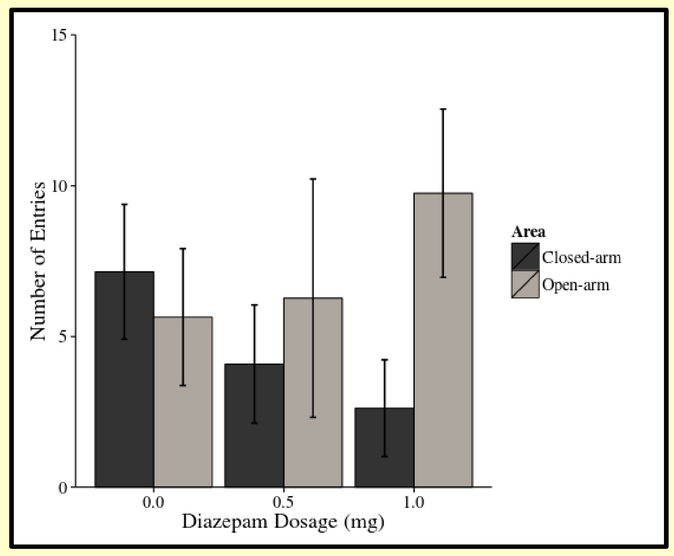


*Figure 1.* A significant difference was revealed in the number of open-arm entries $F(2,21) = 4.114$, $p = .031$ as well as closed-arm entries $F(2,21) = 11.223$, $p < .001$ between drug dosage sizes. The total number of crossings showed no difference, however, between groups $F(2, 21) = 81$, $p = .46$. Rats given 1.0 mg Diazepam performed a greater number of open-arm entries than the saline-injection (0.0 mg) group, $p = .037$. Rats given 1.0 mg Diazepam ($p = .013$) as well as 0.5 mg ($p < .001$) showed more closed-arm entries than the saline group.

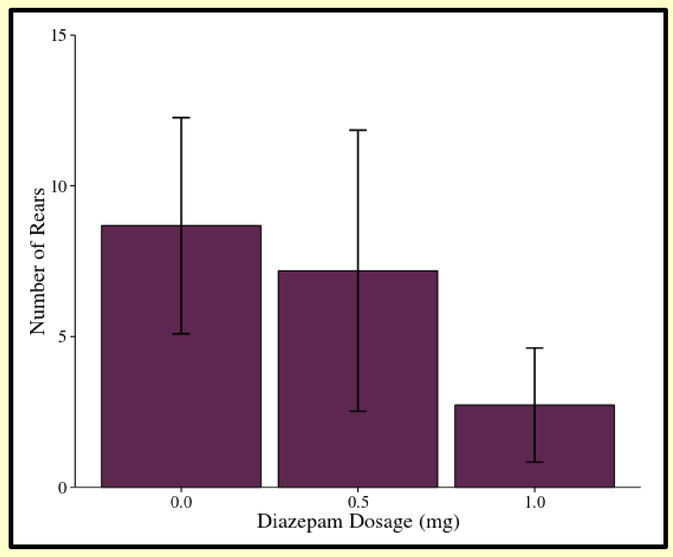


*Figure 2.* The number of rears performed was found to have a significant difference between dose sizes $F(2, 21) = 6.027$, $p = .009$. Rats given 1.0 mg/kg performed significantly fewer rears than the saline group, $p = .008$.

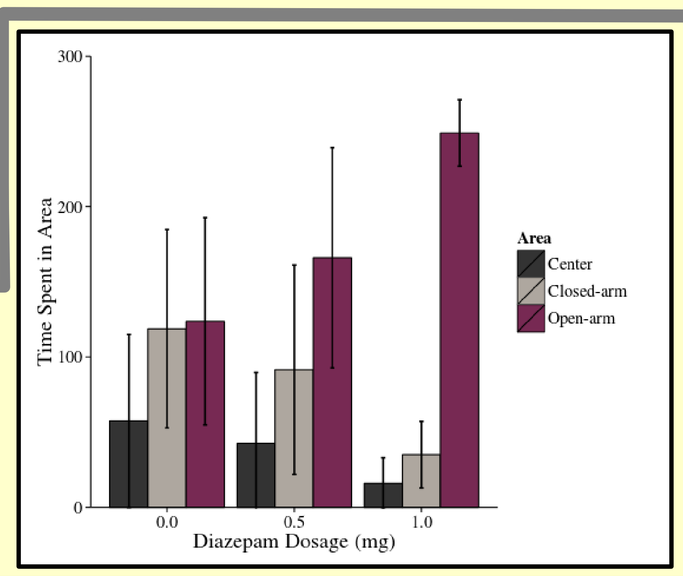


*Figure 3.* Time spent in open-arms $F(2, 21) = 9.209$, $p = .001$, as well as closed-arms $F(2, 21) = 4.526$, $p = .023$, were found to have significant differences across drug-dosage groups. Rats given 1.0 mg/ kg diazepam spent a sifnificantly greater amount of time in open-arms than rats given no drug ($p = .001$) or 0.5 mg/kg diazepam ($p = .028$) . Rats given no diazepam, however, spent significantly more time in closed-arms than did rats given 1.0 mg/kg diazepam, $p = .020$.

## Conclusions

- Entries into and time spent in closed-arms decreased when the size of diazepam dose increased. This was consistent with our hypothesis of a dose-dependent effect on anxiety measures. Rats exposed to an unfamiliar environment tend to stay where they are comfortable – dark, enclosed areas.
- The number of entries into and time spent in open-arms increased with size of diazepam dose. These findings were consistent with our hypothesis as well. Rats which were given diazepam showed exploratory, rather than fear type behavior.
- BZP administration has been shown to cause an enhancement of GABA function throughout the brain. As GABA is an inhibitory neurotransmitter, the brain thus slows globally. One area in particular that may be effected by the increased GABA function is the HPA axis, which is responsible for hormonal stress responses. Saline rats may have experienced HPA axis activation in response to a new environment as a stressor. Tendency to stay in closed-arms then can be seen as a either a coping mechanism, or an automatic response to stress.
- Number of rears decreased with the size of diazepam dose. At the same time, no difference was found in total number of crossings. The sedative effect of 0.5 mg/kg and 1.0 mg/kg may have been enough to reduce the number of rears, but not locomotion seen in the total number of crossings. Muscle relaxing effects of diazepam may have caused rears in particular to feel uncomfortable.
- Non-anxiolytic effects (e.g. sedative effects) of benzodyazapines have been shown to be regulated by GABA A receptor alpha type 1.

## References

Bear, M. F., Connors, B. W., & Paradiso, M. A. (2006). *Neuroscience: Exploring the brain*. Lippincott Williams & Wilkins.

Brady, A.M. (2012). Testing anxiety in the rat. Laboratory handout, PSYC 422, St. Mary's College of Maryland, November 2012

McKernan, R. M., Rosahl, T. W., Reynolds, D. S., Sur, C., Wafford, K. A., Atack, J. R., ... & Whiting, P. J. (2000). Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABAA receptor α1 subtype. Nature neuroscience, 3(6), 587-592.