Experiment no. 14

Aim: Study of anxiolytic activity of drugs using rats/mice

References:

1. Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, 14(3), 149-167.
2. Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *European Journal of Pharmacology*, 463(1-3), 3-33.
3. Crawley, J. N., & Goodwin, F. K. (1980). Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacology Biochemistry and Behavior*, 13(2), 167-170.
4. Kulkarni, S. K. (1999). *Handbook of Experimental Pharmacology*. Vallabh Prakashan.

Objective:

To evaluate the anxiolytic effects of drugs using common behavioral assays in rats/mice.

Materials and Methods:

Materials:

1. Rodents (e.g., mice or rats)
2. Anxiolytic drugs (e.g., Diazepam, Buspirone)
3. Behavioral assays (Elevated Plus Maze, Open Field Test, Light/Dark Box)
4. Control solution (e.g., saline)
5. Anesthetic agents (if required)
6. Personal protective equipment (gloves, lab coat, goggles)
7. Stopwatch
8. Data recording sheets

Method 1: Elevated Plus Maze (EPM)

1. **Preparation of Animals:** Acclimate the rodents to the laboratory environment for at least one hour before the experiment. Handle the animals gently to minimize stress.
2. **Elevated Plus Maze Setup:** The EPM consists of two open arms (50 x 10 cm) and two closed arms (50 x 10 x 40 cm) extending from a central platform (10 x 10 cm), elevated 50 cm above the floor.
3. **Baseline Observations:** Place each rodent in the central platform of the EPM facing an open arm. Allow the rodent to explore the maze for 5 minutes and record baseline activity (time spent in open arms, time spent in closed arms, and number of entries into each).
4. **Drug Administration:** Administer the test drug intraperitoneally or orally, depending on the experimental design. Administer a control solution (e.g., saline) to the control group.
5. **Behavioral Observation:** 30 minutes after drug administration, place each rodent back on the EPM and allow it to explore for 5 minutes. Record the time spent in open arms, time spent in closed arms, and number of entries into each.

Sample Result Table:

Group	Time in Open Arms (s)	Time in Closed Arms (s)	Open Arm Entries	Closed Arm Entries
Control	50	250	5	15
Drug Treated	120	180	12	10

Method 2: Open Field Test (OFT)

1. **Preparation of Animals:** Acclimate the rodents to the laboratory environment for at least one hour before the experiment. Handle the animals gently to minimize stress.
2. **Open Field Setup:** The open field apparatus consists of a large square arena (e.g., 100 x 100 cm) with high walls (40 cm).
3. **Baseline Observations:** Place each rodent in the center of the open field and allow it to explore for 5 minutes. Record baseline activity (time spent in center, time spent in periphery, number of entries into the center, and total distance traveled).

4. **Drug Administration:** Administer the test drug intraperitoneally or orally, depending on the experimental design. Administer a control solution (e.g., saline) to the control group.

5. **Behavioral Observation:** 30 minutes after drug administration, place each rodent back in the open field and allow it to explore for 5 minutes. Record the time spent in the center, time spent in the periphery, number of entries into the center, and total distance traveled.

Sample Result Table:

Group	Time in Center (s)	Time in Periphery (s)	Center Entries	Total Distance Traveled (cm)
Control	30	270	3	2000
Drug Treated	90	210	9	2500

Method 3: Light/Dark Box Test

1. **Preparation of Animals:** Acclimate the rodents to the laboratory environment for at least one hour before the experiment. Handle the animals gently to minimize stress.

2. **Light/Dark Box Setup:** The light/dark box consists of two compartments, one brightly lit (light compartment) and one dark (dark compartment), connected by a small doorway.

3. **Baseline Observations:** Place each rodent in the light compartment and allow it to explore for 5 minutes. Record baseline activity (time spent in light compartment, time spent in dark compartment, and number of transitions between compartments).

4. **Drug Administration:** Administer the test drug intraperitoneally or orally, depending on the experimental design. Administer a control solution (e.g., saline) to the control group.

5. **Behavioral Observation:** 30 minutes after drug administration, place each rodent back in the light compartment and allow it to explore for 5 minutes. Record the time spent in the light compartment, time spent in the dark compartment, and number of transitions between compartments.

Sample Result Table:

Group	Time in Light (s)	Time in Dark (s)	Transitions between Compartments
Control	60	240	5
Drug Treated	150	150	15

Discussion:

1. **Elevated Plus Maze:** Anxiolytic drugs typically increase the time spent in open arms and the number of entries into open arms.
2. **Open Field Test:** Anxiolytic drugs typically increase the time spent in the center, the number of entries into the center, and the total distance traveled.
3. **Light/Dark Box Test:** Anxiolytic drugs typically increase the time spent in the light compartment and the number of transitions between compartments.
4. **Comparative Analysis:** Compare the control and drug-treated groups to assess the anxiolytic efficacy of the test drugs. Effective anxiolytics will show increased exploratory behavior in less anxiety-inducing areas (open arms, center of the open field, light compartment).

Conclusion:

The experiments using the EPM, OFT, and Light/Dark Box provide robust models to evaluate the anxiolytic effects of drugs in rodents. Understanding these effects is crucial for developing effective treatments for anxiety disorders.

Precautions:

- Ensure ethical treatment of animals as per institutional guidelines.
- Handle animals gently to minimize stress and variability in results.
- Maintain consistent environmental conditions to ensure reliable results.