EXPERIMENT NO.: 14 **DATE:**

AIM: TO STUDY THE ANXIOLYTIC ACTIVITY OF DRUGS USING RATS/MICE.

INTRODUCTION:

- Anxiety is chronic condition characterized by an excessive and persistent sense of apprehension with physical symptoms such as sweating, palpitations, and feelings of stress. Anxiety disorders have biological and environmental causes.
- Anxiety is a commonly experienced emotion. It is an uncomfortable feeling of apprehension or fear coupled with sensations of physical arousal.
- If anxiety is excessive or interferes with functioning, it is considered a pathologic anxiety disorder.
- Anxiety is different from fear due to its cognitive component (i.e. fear of the future)
- Anxiety can be helpful and adaptive (e.g. anxiety about giving lectures!)
- An anxiety disorder may be a primary disorder or it may occur secondary to medical causes or substances (e.g., medications or illicit substances); it may occur as a response to acute stressors or it may be associated with another psychiatric disorder.

Types of Anxiety Disorders:

1) Generalized Anxiety Disorder
2) Panic Disorder
3) Phobic Disorders
   - Agoraphobia, Acrophobia,Aquaphobia
4) Obsessive-Compulsive Disorder
5) Post-Traumatic Stress Disorder

MODELS FOR ANTIANXIETY ACTIVITY FOR RATS/MICE

1. ANTI-ANXIETY TEST (LIGHT-DARK MODEL):

Purpose and Rationale

- Crawley and Goodwin (1980) Crawley (1981) described a simple behavior model in mice to detect compounds with anxiolytic effects. Mice and rats tend to explore a novel environment.
- In a two chambered system, where the animals can freely move between a brightly open field and a dark corner.
- They show more crossings between the two chambers and more locomotor activity after treatment with anxiolytics.
- The numbers of crossings between the light and dark sites are recorded.

Procedure:

- The testing apparatus consists of a light and a dark chamber divided by a photocell-equipped zone.
- A animal cage, 44 × 21 × 21 cm, is darkened with black spray over one-third of its surface.
- A partition containing a 13 cm long × 5 cm high opening separates the dark one third from the bright two thirds of the cage.
- An electronic system using four sets of photocells across the partition automatically counts movements through the partition and clocks the time spent in the light and dark compartments.
- Naive male mice or rats are placed into the cage.
- The animals are treated 30 min before the experiment with the test drugs or the vehicle ntraperitoneally and are then observed for 10 min.
- Groups of 6–8 animals are used for each dose.

Evaluation:

- Dose-response curves are obtained and the number of crossings through the partition between the light and the dark chamber are compared with total activity counts during the 10 min.

2. ANTICIPATORY ANXIETY IN MICE:

Purpose and Rationale:

- When group-housed mice are removed one by one from their home cage, the last mice removed have always higher rectal temperatures than those removed first .
- This phenomenon is interpreted as being caused by anticipatory fear for an aversive event.
- This test is thought to be a model of anticipatory anxiety.

Procedure:

- Groups of 18 male albino Swiss mice weighing 25–30 g are used.
- Test drugs or standard (diazepam) or solvent are administered orally in various doses to groups of 18 mice prior to the test.
- Thirty min later, the first 3 mice are removed from the cage and the rectal temperature measured by inserting a silicone lubricated thermistor probe (2 mm diameter) for 2.5 cm into the rectum.
- The average temperature of these 3 mice is taken as basal value.
- thereafter body temperature is determined in the remaining three animals.
- The difference of the mean value of these mice and the basal values is calculated as increase. Vehicle treated test groups display increases of 1.1 to 1.3 °C.

Evaluation:

- The mean increase values of treated groups ±SEM are compared by ANOVA statistics with the controls.

3. SOCIAL INTERACTION IN RATS:

Purpose and Rationale:

- In an unfamiliar and brightly lit environment, the normal social interaction of rats (e.g. sniffing, nipping, grooming) is suppressed.
- Anxiolytics counteract this suppression.

Procedure:

- Male Sprague-Dawley rats (225–275 g body weight) are housed in groups of 5 animals.
- The apparatus used for the detection of changes in social behavior and exploratory behavior consists of a open-topped box (51 × 51 cm and 20 cm high) with 17 × 17 cm marked areas on the floor.
- One hour prior to the test, two naive rats from separate housing cages are treated with the test compound orally. They are placed in to the box and their behavior is observed over a 10-min period by remote video recording.
- Two types of behavior can be noted:
    - Social interaction between the animals is determined by timing the sniffing of partner, crawling under or climbing over the partner, genital investigation of partner.
    - Exploratory motion is measured as the number of crossings of the lines marked on the floor of the test box.
- Six pairs are used for each dose.

Evaluation:

- The values of treated partners are compared with the data from 6 pairs of untreated animals using single factor analysis of variance followed by Dunnett'st-test.

4. ELEVATED PLUS MAZE TEST:

Purpose and Rationale:

- Out of many possibilities to modify maze tests e.g. water maze, the Y-mace, the radial maze and the elevated plus maze have found acceptance in many laboratories.
- The test has been proposed for selective identification of anxiolytic and anxiogenic drugs.

- Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration time; anxiogenic compounds have the opposite effect.

Procedure

- The plus-maze consists of two open arms, 50 × 10 × 40 cm, and two enclosed arms, 50 × 10 × 40 cm, with an open roof, arranged so that the two open arms are opposite to each other.
- The maze is elevated to a height of 50 cm. The rats (200–250 g body weight) are housed in pairs for 10 days prior to testing in the apparatus.
- During this time the rats are handled by the investigator on alternate days to reduce stress.
- Groups consist of 6 rats for each dose.
- Thirty min after i.p. administration of the test drug or the standard, the rat is placed in the center of the maze, facing one of the enclosed arms.
- During a 5 min test period the following measures are taken:
- the number of entries into and time spent in the open and enclosed arms.
- the total number of arm entries.

Evaluation

- Motor activity and open arm exploratory time are registered. The values of treated groups are expressed as percentage of controls. Benzodiazepines and valproate decrease motor activity and increase open arm exploratory time

5. WATER MAZE TEST:

Purpose and Rationale:

- Spatial learning of rats can be tested in a water maze

Procedure:

- The water maze consists of a circular tank with 100 cm diameter and a wall 20 cm above the water level.
- A circular platform (9 cm diameter) is hidden 2 cm below the water level.
- The water is made opaque using titanium dioxide suspension and is kept at about 23 °C during the experiment.
- Training takes place on three consecutive days, with the rats receiving 4 consecutive trials per day with an trial interval of 6–10 min.
- Latency to find the platform is measured as the time of placement of the rat in the water to the time it finds the platform.
- If the animal fails to find the platform in any trial within 3 min it is placed on it for 10 s.

Evaluation:

- The platform is removed and the time spent in the target quadrant (the quadrant in the center of which the platform has been located) and the number of annulus crossings across the actual location where the platform has been located in the first 60 s of exposure are measured.
- The time to the first annulus crossing is taken as a measure of performance of trial.
- Buspirone as well as benzodiazepines increase the latency to find the platform in the training period and impair the number and the time of annulus crossings.

6. STAIRCASE TEST:

Purpose and Rationale:

- When introduced into a novel environment, rodents experience a conflict between anxiety and exploratory behavior manifested by increased vigilance and behavioral activity.
- In the staircase paradigm, step-climbing is purported to reflect exploratory or locomotor activity, while rearing behavior is an index of anxiety state.
- The number of rearings and steps climbed are recorded in a 5 min period.
- The dissociation of these parameters is considered to be characteristic for anxiolytic drugs.

Procedure:

- For experiments with mice the staircase is composed of five identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep.
- male mice with a weight between 18 and 24 g are used. Each animal is used only once.
- The drug or the standard is administered orally 1 h or 30 min subcutaneously before the test. The animal is placed on the floor of the box with its back to the staircase.
- The number of steps climbed and the number of rears are counted over a 3-min period.

Evaluation:

- Twelve mice are used for the untreated control groupnm and for the group receiving the standard.
- The average number of steps and rearings of the control group is taken as 100%.
- The values of treated animals are expressed as percentage of the controls.

7. CORK GNAWING TEST IN THE RAT:

Purpose and Rationale:

- Cork gnawing behavior in the rat has been proposed as a screening method for buspirone-like anxiolytics.

Procedure:

- Adult male Evans rats serve as subjects.
- They are housed 4 per cage on a regular light/dark cycle with free access to food and water
- For the test session one animal is placed in a stainless steel cage with wire mesh bottom.
- A session consists of placing the subject in the test cage with a cork stopper weighing between 2–3 g for 30 min.
- Initially, the amount gnawed is relatively high and variable within and between subjects. After 30 training sessions, the amount is low and stabilized.
- The test compounds are injected 30 min before the test and food is withdrawn.
- The average cork loss during the previous control days is taken as baseline and the amount after drug treatment is expressed as percentage of baseline.
- Buspirone-related compounds as well as benzodiazepines and meprobamate show a dose dependent increase of cork gnawing.

Evaluation:

- Each cork is weighed to the nearest 0.01 g before and after the session.

8. SCHEDULE INDUCED POLYDIPSIA IN RATS:

Purpose and Rationale:

- Food deprived rats exposed to a procedure in which food is delivered intermittently will drink large amounts of water if given the opportunity to do so.
- This behavioral phenomenon is termed schedule-induced.

Procedure:

- Male Wistar rats weighing 180–250 g are individually housed at a 12 h/12 h light/dark cycle for a 1 week acclimation period with free access to food and water.
- Then they are placed on a restricted diet which maintains 80% of their free feeding body weight.
- To induce polydipsia, rats are placed in test chambers housed in sound attenuated boxes where a pellet dispenser automatically dispenses two 45 mg pellets on a fixed time 60-s feeding schedule over a 150 min test session.
- Water is available at all times in the test chambers.

- After 4 weeks exposure to the 60s feeding schedule, approximately 80% of the rats meet the pre-determined criterion for water consumption (greater than 60 ml water per session) and are considered to have polydipsic behavior.

Evaluation:

- The experimental data comparing the effects of chronic administration of compounds on schedule-induced polydipsia are analyzed with the Mann Whitney U-test.

9. FOUR PLATE TEST IN MICE:

Purpose and Rationale

- The four plate test in mice has been used for the rapid screening of minor tranquilizers.

Procedure:

- The test box has the shape of a rectangle (25 × 18 × 16 cm).
- The floor is covered with 4 identical rectangular metal plates (8 × 11 cm) separated from one another by a gap of 4 mm.
- The plates are connected to a source of continuous current which applies to 2 adjacent plates a mild electrical shock of 0.35 mA for 0.5 s. This evokes a clear flight reaction of the animals.
- Adult male Swiss albino mice, weighing 17 to 23 g, are randomly divided into different groups.
- Thirty min before the test the animals are injected intraperitoneally with the test drug or the vehicle.
- At the beginning of the test, the mouse is gently dropped on to a plate and is allowed to explore the enclosure for 15 s.
- After this, every time the animal crosses from one plate to another, the experimenter electrifies the whole floor for 0.5 s, which evokes a clear flight-reaction of the mouse.

Evaluation:

- The number of times the apparatus is electrified is counted each minute for 10 min.
- The delivery of shocks decreases dramatically the motor activity.
- The number of shocks received during the first min is taken as parameter. This number is increased by minor tranquilizers, such as benzodiazepines.

10. FOOT SHOCK INDUCED FREEZING BEHAVIOR IN RATS:

Purpose and Rationale:

- Footshock-induced freezing behavior in rats has been proposed as a model for anxiolytics.

Procedure:

- Male Sprague-Dawley rats with a weight between 200 and 350 g are used.
- The animals receive a single i.p. injection of the test compound or the vehicle 30 min prior to being placed in a standard conditioning chamber (e.g., Coulborn Instruments) for a 6.5 min session.
- 2 and 2.5 min after the start of the session, a footshock (0.5 mA, 0.5 s) is delivered through the grid floor of the chamber. Following exclusive behaviors are observed:
- Freezing: immobility with rigid body posture
- Sedated posture: sitting or sleeping
- Small exploratory movements: movements involving the torso or front paws only, vertical movements of the head, or sniffing.
- Locomotion: activity involving hind paws, grooming or rearing. Frequency of rearing is also counted. All behaviors are monitored for the entire 6.5 min session.

Evaluation:

- Duration of foot-shock induced freezing after the second shock is taken as the critical parameter.
- Time spent in freezing posture after administration of test compounds is compared with the controls.

11. mCPP INDUCED ANXIETY IN RATS:

PURPOSE AND RATIONALE

- The metabolite of the antidepressant drug trazodone 1- (3-chlorphenyl) piperazine (=mCPP), classified as 5- HT1C agonist or 5-HT1B/2C agonist has been shown to be anxiogenic .
- Antagonism against these symptoms has been proposed as a screening model for anxiolytic drugs.

Procedure:

- Male Sprague Dawley rats (220–250 g) are housed in groups of 6 under a 12 h light/dark cycle with free access to food and water.

mCPP - induced locomotion:

- Rats are used.

- They are dosed either orally 1 h, or i.p. 30 min before the locomotion test with test compound or vehicle, and injected 20 min before the test with 7 mg/kg mCPP i.p. or saline in groups of four.
- At 0 h they are each placed in automated locomotor activity cages.
- locomotion is recorded by means of alternately breaking two photocell beams traversing opposite ends of the box 3.9 cm above floor level.

mCPP-induced hypophagia:

- Rats are individually housed on day 1 and on day 3 they are deprived of food.
- Twenty-three hours later, the are orally treated with the test drug or vehicle.
- Forty min later, they are given 5 mg/kg mCPP or saline i.p.
- After a further 20 min, weighted amounts of their normal food pellets are placed in their food hoppers and the amount remaining after 1 h is measured.

Evaluation:

- The effect of the test compound on mCPP-induced hypolocomotion is determined by one-way ANOVA and Newman-Keuls test. The dose producing 50% disinhibition of mCPP is also estimated.
- Feeding test data are subjected to one-way ANOVA and Dunnett's test.

12. ACOUSTIC STARTLE RESPONSE IN RATS:

Purpose and Rationale:

- The acoustic startle reflex is a relatively simple behavior that occurs naturally in mammals.
- It consists of a series of rapid movements beginning at the head and contraction and extension of major muscle groups .
- Startle response can be used to determine sites and mechanisms of drug action

Procedure:

- Male Wistar rats weighing about 200 g are used.
- Acoustic startle reflexes are measured in a specially build apparatus, e.g., Coulborn Instruments Acoustic Response Test System.
- The animals are individually placed in 8 × 8 × 16 cm open air cages that restrict locomotion but do not immobilize the animal.
- Sound-attenuating acoustic chamber used when sound is produced physical movement is measured.

- Data are recorded automatically by an interfaced microcomputer. Pre-tests are performed with all animals to obtain control values.
- The animals are treated 2 h prior the experiment with test drugs or vehicle given orally or subcutaneously.

Evaluation:

- The results are given as percentage of the change, related to the values obtained in the pre-test and assessed by a one-way ANOVA, followed by Dunnett's test when appropriate.

13. UNCONDITIONED CONFLICT PROCEDURE (VOGEL TEST):

Purpose and Rationale:

- Described a simple and reliable conflict procedure for testing anti-anxiety agents.
- Thirsty, naive rats were administered shocks while licking water.

Procedure:

- The apparatus is a clear Plexiglas box (black in color) ,The entire apparatus has a stainless-steel grid floor.
- A water bottle with a metal drinking tube is fitted to the outside of the small compartment, so that the tube extended into the box at a height 3 cm above the grid.
- Rats lick in constant rate of 7 licks per sec. A drinkometer circuit is connected between the drinking tube and the grid floor of the apparatus, so that the rat completes the circuit whenever it licks the tube.
- Shock is administered to the feet of the animal.
- Thirty min after i.p inj, the rat is placed in the apparatus and allowed to find the drinking tube and to complete 20 licks before shock is administered.
- The rat controls shock duration by withdrawing from the tube.
- A 3-min timer is automatically started after the termination of the first shock. During the 3-min period, shocks are delivered following each twentieth lick.
- The number of shocks delivered during the 3-min session is recorded for each animal.

Evaluation:

- The number of shocks received after treatment is compared with untreated animals. Benzodiazepines increase dose-dependent the number of shocks. Barbiturates at low doses active in this test.

14. NOVELTY-SUPPRESSED FEEDING:

Purpose and Rationale:

- Placing a hungry rat into an unfamiliar environment with access to food results in a suppression of feeding behavior relative to the condition when the test environmentis familiar.
- This effect has been termed hyponeophagia and occurs because of the novelty of the test environment. The avoidance of novel foods is termed food neophobia.
- Both hyponeophagia and food neophobia have been assumed to measure anxiety by eliciting a conflict situation arising from a fear of the novel setting and foods, and the drive to eat.

Procedure:

- The testing apparatus consists of individual Plexiglas open fields, 76 × 76 × 46 cm.
- Thirty Purina lab chow pellets are placed in a pile directly in the center of the open field. Animals are handled for 3 weeks prior the behavioral testing. Forty-eight hours prior to testing.
- all food is removed from the home cage, although water is still available. 1 One h prior to testing, animals receive an intraperitoneal injection of test drugs or vehicle.
- At the time of testing, the animals are placed into individual open fields containing the food, and the latency to begin eating is measured..
- If the animal has not eaten within 720 s, the test is terminated and the animal is assigned a latency score of 720 s.

Evaluation:

- An anxiolytic effect is defined as a significant decrease in mean latency to begin eating compared with vehicle controls.

15. SHOCK PROBE CONFLICT PROCEDURE:

Purpose and Rationale:

- Rats being placed in a novel test environment containing a probe.
- Number of times that the animal makes physical contact with it, is reduced when the probe is electrified.
- Rats treated with anxiolytics continue to touch the electrified probe.

Procedure:

- Apparatus: The test environment consists of a Plexiglas chamber, measuring 40 × 40 × 40 cm, and having a metal grid floor.

- Whenever the animal touches both wires simultaneously with some part of its body, a DC current flows through the animal.
- Sixty min after treatment with saline or test substance, the animal is placed in a back corner of the testbox facing away from the probe.
- The number of responses the animal makes during the subsequent 5-min episode is counted.

Evaluation:

- Dose-response curves can be established for various drugs at different shock intensities.
- The Mann-Whitney U-test is used to evaluate differences between experimental conditions.
- To control whether a drug treatment increases responding above the saline control level, an one-tail t-test is used.

16. ULTRASOUND INDUCED DEFENSIVE BEHAVIOR IN RATS

Purpose and Rationale:

- Production of ultra-sonic calls in the 20–27 kHz range are used,
- Rats display specific defence behavior as a part of their natural survival strategy.

Procedure:

- The apparatus consists of a circular open field 75 cm in diameter, 46 cm high walls, with a video camera suspended above.
- Locomotor behaviors are recorded and analyzed using a computer automated tracking system capable of rapid movements. This allows the ultrasound induced change in locomotor behavior to be quantified in maximum speed, average speed and distance traveled by the animals.
- Animals are placed in the test arena 20 min after intraperitoneal injection of drug or vehicle and locomotor activity is measured.
- After 2 min they are exposed to a 1-min, 20 kHz, square wave ultrasound tone followed by a further 2 min without sound. This procedure is repeated for each intensity with a 1-min interval.
- Locomotor activity values are then calculated for the maximum speed, average speed and total distance traveled through out the 5-min test period and expressed as a series of 15–20-s .

Evaluation:

- Maximum speed is analyzed using a two-way ANOVA.

- Significant interactions between treatment and time are followed by one-way ANOVAs for individual time points with post-hoc Duncan's new multiple range test.

17. ANXIETY/DEFENSE TEST BATTERY IN RATS:

Purpose and Rationale

- It is the procedures designed to assess the defensive reactions of rats to a natural predator, the cat.
- The primary measures, taken both during and after cat presentation, include movement arrest and risk assessment and the inhibition of non-defensive behaviors .

Procedure:

- The test apparatus for both the proxemics/activity and eat/drink procedures consists of two parallel subject chambers (53 × 20 × 25 cm).
- Subject movements are monitored by five photocells mounted at equal distances
- The initial study assesses the effects of cat exposure onproxemics/activity, followed 7 days later by analysis of eat/drink behavior during and after cat exposure. Both procedures are carried out under dim red light.

Proxemic/activity testing:

- Rats are individually placed in each compartment of the test apparatus. Following a 5-min pre-cat period, the cat is introduced to the cat compartment for 5 min. Following removal of the cat,,behavior is recorded for a further 15 min post-cat period,

Eat/drink testing:

- Rats are individually given 2 g of finely crushed chocolate cereal on the 2 days after the proxemic/activity test, to familiarize them with this highly preferred food.
- In order to induce a mild water deprivation, water bottles are removed, 24 h prior to eat/drink testing.
- Same as proxemic / activity testing butMeasures of eating frequency and duration, and drinking frequency are taken for the cat and post-cat periods.

Evaluation:

- The data are analyzed by analysis of variance (ANOVA).