Animal Model of Anxiety

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1 Introduction

Anxiety is defined as state of unpleasant and uneasiness or discomfort experienced on exposure toward threat or painful stimuli both in humans and animals. It is cumulatively caused by increased activity of neuroendocrine and autonomic nervous system. Also, it is a state of behavioral disturbance, that is, sense of unrealistic worry about everyday life situations. Animal models for anxiety-related behavior are based on the assumption that anxiety in animals is comparable to anxiety in humans. Being anxious is an adaptive response to unfamiliar environmental conditions, especially during unconformity with danger or threat. Human anxiety disorders are broadly grouped according to symptomology and responsiveness to pharmacological and psychological treatment. Generalized anxiety disorder and panic disorder are the two primary classifications of pathological anxiety in humans. In generalized anxiety disorder, the peoples experience unrealistic worry about everyday life situations, which make it different from panic disorder. In contrast, panic attacks mainly indicate the primary symptoms of panic disorder with intense fear, palpitation, and sweating, etc. These events are characterized as sudden, extreme fear accompanied by autonomic nervous system arousal (Battaglia et al. 2005).

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In response to the types of stimuli which cause anxiety, the behavioral models are classified into two types, i.e., either conditioned or unconditioned. In the conditioning models, the minor stimuli are used like deprivation of animal from food and water or giving foot electric shock, etc., but unconditioned models (spontaneous) have higher degree of ecological validity. They are also less susceptible to be arising from interference with learning/memory, hunger/thirst, or nociceptive mechanisms. These animal models provide a powerful contribution to the area of research related to anxiety at the clinical, industrial, and scientific levels. In this, the individual susceptibility difference among the animals toward anxiogenic stimuli and variable responses to different types of threats can easily be modelled in animals. It is easy to analyze the basic physiological mechanisms underlying fear in rodents because of the similar mechanisms operating in humans provide a degree of face validity for these paradigms. The rodents mainly show these responses which may be appropriate and adaptive for the current conditions, but in humans, the anxiety disorders constitute maladaptive or pathological responses to the existing situation. Further, to explore the neuroanatomy and neurochemistry involved in fear in rodents toward both conditioned and unconditional fear could offer important insights into effective targets for novel pharmacological treatment. However, it is very difficult to correlate biologically the animal studies with human behavior because: (i) the difference between human's and non-human's nervous systems; (ii) the difficulty in determining analogous behaviors among species; and (iii) the need to extrapolate the results from animals to humans.

2 Classification of Animal Models of Anxiety (Fig. 1)

2.1 Conditioned Response

2.1.1 Geller–Seifter Conflict

Principle: The Geller–Seifter conflict model is commonly used from the last few decades for the evaluation of anxiolytic drugs. In this model, multiple operant schedules are used by providing shock after the food cues (Howard et al. 1990). These food cues increase the reinforcement, and shock act as signal to confirm the behavior of the animal, i.e., if the animal is in anxiety state it does not respond to shock signals.

Procedure:

- The rats with body weight 180–250 are housed individually.
- The rats are trained in the chamber which is operated by a lever to obtain food.

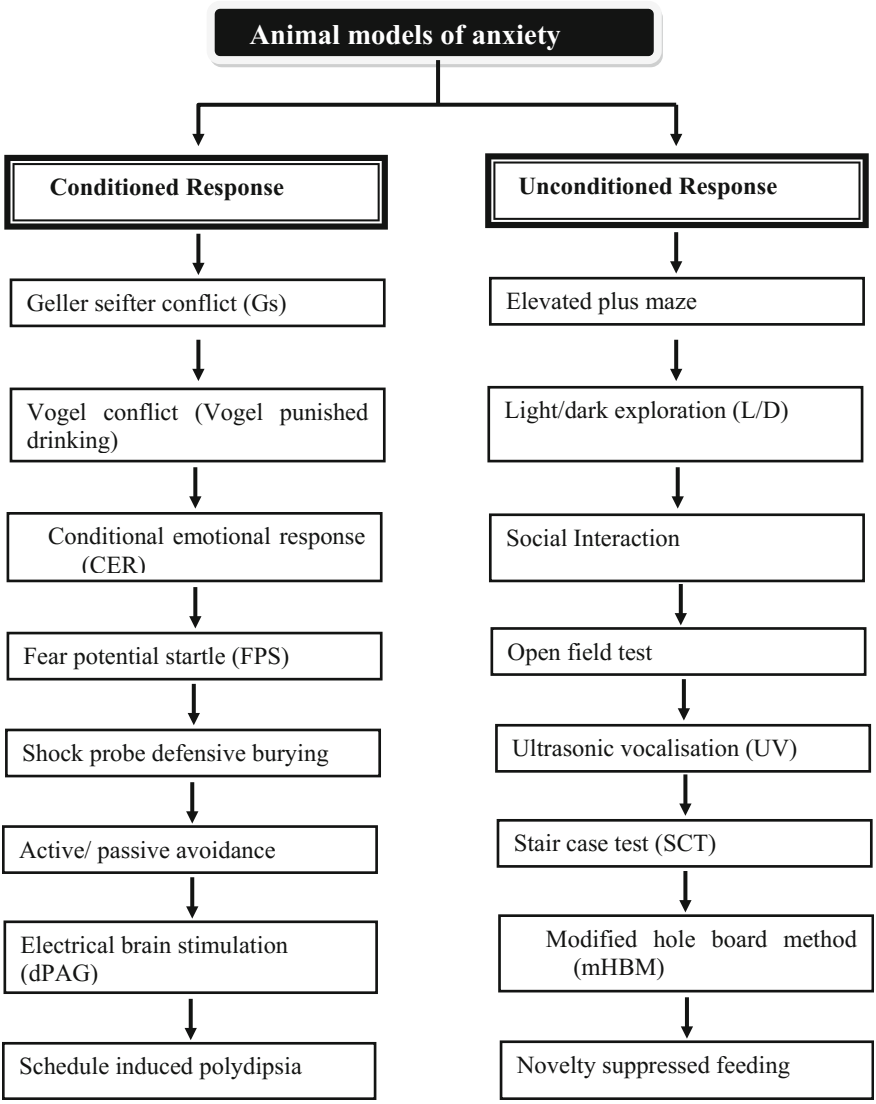

Fig. 1 Classification of animal models of anxiety

- Auditory cue in the form of signal is provided to increase the reinforcement contingencies.
- After the auditory cues, i.e., during the next session, food is available to the animals along with foot shock.
- The test procedure consists of four 15-min non-shock variable interval segments in which the reinforcement is available on a restricted basis.

- The whole test procedure consists of multiple schedule of reinforcement to evaluate the anxiolytic action of drug at different intervals.
- To analyze the drug the auditory cues: first, the response of reinforced is given at irregular intervals but afterward, every response is simultaneously reinforced (signalled by a different signal) and punished by the delivery of inescapable electro-shock.
- The response to these signals can be suppressed by administration of anxiolytics.

Advantages of G-S conflict test

1. This method has selectivity for anxiolytic drugs showing no effects of other classes of psychotropic drugs.
2. G.S method is useful for evaluation of chlordiazepoxide, diazepam, meprobamate, phenobarbital, and pentobarbital.
3. It is a suitable method for repeated drug testing.
4. Once the subjects have learned the tasks in the Geller–Seifter paradigm response rates in all operant components remain relatively stable over long periods (Willner et al. 1992). This makes the Geller–Seifter conflict a suitable test for repeated drug testing in order to demonstrate reliable and repeatable responses to anxiolytics over time in individual subjects.

Disadvantages of G-S conflict test

1. A long period of training (one to several weeks) until the animals reaches a stable baseline response to the conflict component as well as the necessity for long-term food restriction.
2. Sometime animals may die due to over electric shock.

2.1.2 Vogel Conflict

Principle: It is also called Vogel punished drinking or Vogel water-lick conflict test. The Vogel water-lick conflict is a modification of the Geller–Seifter conflict paradigm that was established to eliminate the long periods of training. It is a commonly used method to study anti-anxiety drugs in which water cues are provided for a short interval (Safi et al. 2006).

Procedure:

- Male Wistar rats of body weight 180–250 g are selected and are deprived of water for 24 h prior to the start of first training session.
- The first training session is consisted of two 3-min periods in which the number of unpunished licking spells is recorded.
- Prior to drug administration that is after the competition of first training session, the animals are placed back in the box for conflict test.

- Now the animals are administered with drug, moved to the apparatus to start the trial consisted of two 3-min periods in which rat completed 20 licks received first shock.
- After every 20 unpunished licks, 1 mA current is provided between the grid floors and drinking for the subsequent licking.
- The animals are shocked with current for fixed cycle of 3 mins. The animals which show 50% suppression of licking during second session in comparison with first trial are selected for the study.
- The drugs are to be administered after second trial competition, and again the animals are placed into their respective cages with availability of water.
- The total test time per rat is 12 min per week (Fig. 2)

Advantages of Vogel water-lick conflict

1. It is a modified form of Geller–Seifter conflict and required less training time to evaluate anti-anxiety drugs.
2. It also responds to some non-anxiolytic drugs, producing false-negative results, but antidepressants produce inconsistent results in these models.
3. By this method, we can compare the anxiolytic efficacy of different drugs.

Disadvantages of Vogel water-lick conflict

The major limitation of using the Vogel water-lick conflict is the lack of a systematic analysis of drug effects on non-conflict behavior. Later, the modified form of this method has improved replicability by preselection of subjects that lick water but are sensitive to shock induced suppression.

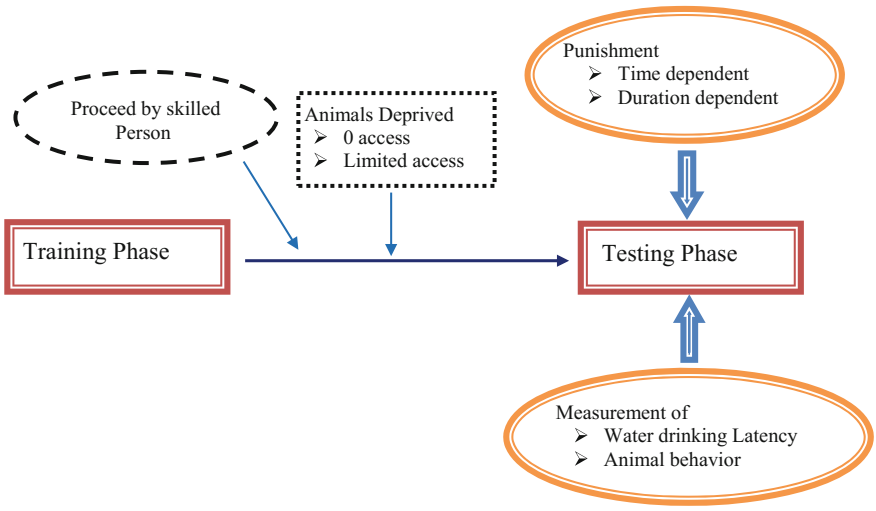

Fig. 2 Diagrammatic representation of Vogel punished drinking

2.1.3 Conditioned Emotional Response (CER)

Principal: The CER is the simplest method and was firstly discovered by Ivan Pavlov used for testing efficacy of anti-anxiety drugs. In this model, the conditional stimuli of food cues are provided with electric shock after giving training to the animals. The electric stimuli are provided to animals at different time interval of training along with food pellets.

Procedure:

- The experiment is conducted in apparatus consisted of four identical operant chambers. The floor consists of electrifiable grid, and the side walls are fitted with a single bar containing a food tray under it.
- During the preliminary training phase, each chamber is provided with 45 mg food pellets for a 1-min variable interval schedule (IV), also called magazine training.
- Now immediately after preliminary training, continuous reinforcement schedule is given with a delivery of 120 food pellets in a single session.
- At the end of training session, six daily 2-h sessions of bar pressing under a 2.5-min variable interval of food reinforcement schedule are given to the animals.
- This result in acquisition of stable bar-pressing behavior for food, and the numbers of bar presses emitted by each rat in 3-min periods on the 6th day are noted with achieving a conditional stimulus (CS).
- The conditional response consists for 3-min period of 80 dB, but noise is delivered by permanent magnet speaker placed below the floor of the experimental chamber.
- After this on dummy day conditional stimulus of 0.5 s with shock of 2-mA intensity is given at intervals 14, 48, 72, and 79 min after beginning of session.
- The procedure was repeated for three consecutive days. The magnitude of CES is measured by the "suppression ratio."

Advantages

1. The CER is a simple behavioral paradigm in which organisms learn to predict aversive events.
2. It evaluates the clinical efficacy of anxiolytic on different animals by providing different conditional stimulus.

Disadvantages

1. The main drawback of this method is that the stimuli used are painful and may also induce fear in animals.
2. Highly skilled person is required because the experiment consists of number of training phase intervals.

2.1.4 Fear Potential Startle (FPS)

Principle: In FPS, the fear reactions are used as stimuli of reflux response in animals, and the response are elicited in the form of threatening stimulus (e.g., any object, person, or situation produces feelings of fear). These can also be delivered by a neutral stimulus as a result of fear conditioning. The stimulus used is usually of auditory (e.g., loud noise) or visual (e.g., bright light) type, and startle response measures include eye blink rates and pulse/heart rate.

Procedure:

- The procedure consists of 3 days of startle acclimation in which classical fear conditioning is provided for 1 day followed by a fear-potentiated startle test session.
- In this, the animals are provided with 5-min acclimation period followed by 30 presentations of a 50 ms. After this, noise burst startle stimulus at 95, 105, or 115 dB (10 of each) are to be given in a predetermined pseudo-random order.
- Each startle consists of 15-s inter-trial interval, and these help in easy acclimation of the subjects to the experimental environment and also improve matching subjects into experimental groups.
- Measure the mean "Pre-Fear" startle scores for each subject which are formed by addition of mean startle amplitudes of all the trials over the 3 days.
- Now those animals which pass the Pre-Fear startle amplitudes are administered with different dose conditions for matching the various groups to different conditions.
- After the administration of doses, all the rats are classically fear-conditioned for the four days. During the 5-min acclimation period, foot shock is provided with five pairings of light stimuli.
- Each pairing consisted of 3-s presentation of the light, which co-terminated with the 500 ms (0.6 mA) foot shock, the inter-trial intervals ranged from 60 to 180s in a pseudo-random order.
- Now compare the results of before and after treatment for measuring the efficacy of drugs.

Advantages

1. It provides a direct correlation between the anxiety behavior of animal and anxiety disorder patients as a result of re-exposure to trauma-related stimuli or negative life events.
2. It serves as a "translational bridge" and is the first to use fear-potentiated startle to examine extinction and reinstatement in humans.

Disadvantages

1. Depending upon the signs of fair in animals, it is very difficult to correlate the model to behavior signs of anxiety in humans.
2. Sometimes, the animals may not respond to fear-induced anxiety-like state.

2.1.5 Shock Probe Defensive Burying

Principle: This model was introduced 25 years ago by Pinal and Treit. Defensive burying refers to the typical rodent behavior in which the bedding material is displaced with vigorous material. Due to this, the animals show treading-like movements of their forepaws and shovelling movements of their heads when directed toward a variety of noxious stimuli. In this, animal is exposed toward immediate threat, such as a wall-mounted electrified shock-produce.

Procedure:

- In this, the test apparatus is covered with suitable bedding material and the subjects are confronted with a wire-wrapped probe (Ø = 1 cm; 6–7 cm long). A small hole lies 2 cm above the bedding in one of the test chamber walls.
- The shock source is connected through non-insulated wires of the probe.
- Now during the teat session when the animal touches the probe, they receive an electric shock (manually operated or automatically delivered).
- Observe the animal's behavior manually or recorded on video for a 10–15-min test session.
- During this observation period, all occurring behavioral postures and the parameters are measured for maximum 15 mins.

Advantages

1. Shock probe test is helpful in detecting the neuroendocrine effect in anxiety, because noradrenaline plays a crucial role in emotional behavior in animals and humans.
2. This paradigm not only is suitable for screening potential anxiolytic properties of drugs but also seems to be especially valuable for unravelling the neural circuitry and neurochemical mechanisms involve in anxiety (Reynolds et al. 2001).

Disadvantages

1. This experiment requires a long training session for the proper acclimation of animals to evaluate anti-anxiety effect of drugs.
2. The cut-off time is too long that may increase the mortality rate because assembly is fitted with probe carrying current.

2.1.6 Active/Passive Avoidance

Passive Avoidance

Principle: Passive avoidance task is fear-aggravated test used to evaluate learning and memory in experimental animals. In this procedure, the animals are learned to

avoid noxious event by suppressing a particular behavior when they are exposed to different conditions.

Procedure:

- The apparatus consists of two adjacent Plexiglas compartments of identical dimensions (27 cm × 14.5 cm × 14 cm) with grid floors.
- The floor of the two compartments has been covered with stainless steel bars (2 mm diameter) spaced 1 cm apart. The compartment is illuminated by a 5-W lamp mounted on its wall just below a movable transparent Plexiglas ceiling.
- The animals are allowed to adapt for 10 min period with free access to either the light or dark compartment of the avoidance training box after being placed in a shuttle-box.
- After the two days of adaptation period, the animals are placed into the illuminated compartment.
- To note the latency of learning phase, the sliding door is raised 30 s later.
- Close the door when the animal move into dark compartment, and a 1.5-mA constant current is applied to the fore and hind paws for 3 s.
- Again after 20 s, each animal is removed from the dark compartment and placed into the home cage.
- For the testing of short-term learning, that is, 24 h after receiving foot shock, the animals are placed in the illuminated chamber again.
- After 30 s, the sliding door is raised and latency of entering the dark compartment is recorded again constituting the step-through latency.
- The maximum cut-off time for this procedure is 5 min.

Advantages

1. Passive avoidance is a better behavioral test for learning and memory studies, because it requires little special training of the subjects and also the results are available quickly.
2. It is a simple and fast method for evaluating psychotropic and anxiolytic drugs.

Active Avoidance

Principle: The active avoidance task is a fear-motivated test in which electric current is used as a source of punishment. In this, the animals are learned to predict the occurrence of an aversive event based on the presentation of a specific stimulus in order to avoid the harmful stimuli by actively moving to a different compartment.

Procedure:

- The apparatus used for evaluating active avoidance consists of 3-equal arms like Y-maze (Narwal et al. 2012).

- Prior to the experimentation, the rats are trained in the maze for minimum 30 trials daily for 4 days.
- The conditional stimuli (CS) are provided to the animals by using a 12-W light bulb, whereas unconditioned stimuli (UCS) in the form of 3 mA electrical foot shock.
- Inter-trial interval (ITI) and inter-stimulus interval (ISI) are of 60 s and 5 s, respectively.
- Trained animals left the dark arms and entered into the light arm. If this occurred within the 5 s of ISI, the effort is counted as a conditioned response.

Advantages

1. Active avoidance is useful model for neuropharmacological and electrophysiological studies.
2. This paradigm also takes a less time to access even short-term changes in the performance of animals.

2.1.7 Electrical Brain Stimulation (dPAG)

Principle: Electrical stimulation of the dPAG has been proposed as a model of panic attacks. According to this model, a stepwise increase in the electrical current intensity to stimulate the dPAG produces alertness, then freezing, and finally the panic-like behavior characterized by running and jumping responses.

Procedure:

- In this model, the animals are placed into the experimental cage and the escape threshold is determined by applying electrical stimuli (AC, 60 Hz, 10 s) through the implanted chemitrode.
- The inter-stimulus interval is 10 s, and the current intensity is started at a level of 20 A (peak-to-peak) and is increased by steps of 4 A.
- Apply the electric stimuli until the rat started to run around the circular arena, indicating the escape behavior. Sometimes the animals also show vertical jumps as an indicative of vigorous reaction.
- After observing these behaviors, the application of electrical stimulation to the dPAG is interrupted by the experimenter person.
- The basal escape threshold is defined as the lowest current intensity that evoked escape in three successive trials of electrical stimulation. Animals with basal thresholds above 152 A are excluded from the study.

Advantages

1. This is the best model used to differentiate between panicolytic drugs like clomipramine, fluoxetine and panicogenic drugs like pentylenetetrazole.
2. The use of dPAG model helps us clearly differentiating the anxiety and panic attack.

2.1.8 Schedule-Induced Polydipsia in Rats

Principle: Schedule-induced polydipsia is a behavioral model in which the excessive drinking developed by food-deprived animals exposed to intermittent food reinforcement schedules. This short-term food exposure to the animals at different interval shows better predictivity for analyzing anti-anxiety drugs.

Procedure:

- Firstly, weigh the animals and allocate randomly to one or two groups that is the polydipsia group or the control group.
- After a 1-week acclimatization period, the animals are subjected to 15 preoperative schedule-induced polydipsia tests on weekdays.
- Place the animals in the test chamber with automatic delivery of food (45 mg) pellets on a fixed-time 60 s feeding schedule for 30 min test sessions.
- To assess schedule-induced polydipsia, water intake (g) is measured by weighing the water bottles before and after the 30 min test sessions.
- The testing of the animals is done on every day randomly. The animals which consumed 8 ml water or more are considered to be polydiptic (SIP group).
- Control animals are tested in the same environment but received all the 30 food pellets at once, and they are paired in group with an animal from the SIP group.
- Those animals which do not meet the 8 ml criterion (SIP criterion) after 15 test days are considered resistant or resistant group.

Advantages

1. It is a useful model to study those neuropsychiatric disorders characterized by the presence of compulsive behavior such as obsessive–compulsive disorder (OCD), schizophrenia, and alcohol abuse.
2. SIP provides a bitonic relationship between amount of water drinking and inter-reinforcement interval length.

2.2 Unconditioned Response

2.2.1 Elevated Plus Maze

Principal: The elevated plus maze is a widely used behavioral test for rodents, and it has been validated to assess the anti-anxiety effects of pharmacological agents and steroid hormones. Briefly, rats or mice are placed at the junction of the four arms of the maze, facing an open arm, and entries/duration in each arm is recorded manually or by a video tracking system for 5 min.

Procedure:

- The apparatus 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 (Fig. 3).
- The maze lies at 50 cm height from the ground floor. 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.
- The animals are divided into test and control group. Now 30 min after ip 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:

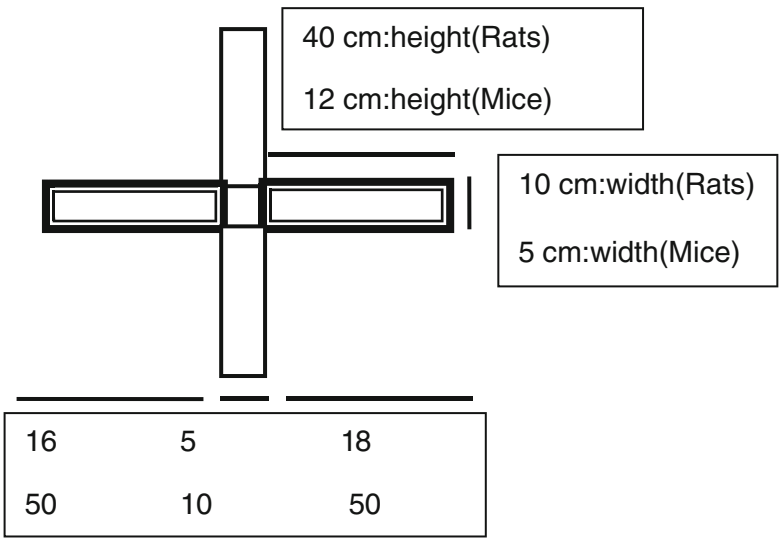

Fig. 3 Elevated plus maze

1. The number of entries into and time spent in the open and enclosed arms.
2. The total number of arm entries.

The procedure is conducted preferably in a sound attenuated area, and the observations are made from an adjacent room via a remote control TV camera or manually.

Advantages

1. Anxiolytic compounds increase open-arm activity, but anxiogenic shows opposite response.

2.2.2 Light/Dark Exploration (L/D)

Principle: The light/dark test is based on the principle that innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors. The drug-induced movement of animal toward light area can be tested which indicate the efficacy of the drug.

Procedure:

- The testing apparatus consists of a light and a dark chamber divided by a photocell-equipped zone. The one-third of the animal cage is darkened with black spray.
- Both the dark one-third and the bright two-thirds of the cage are partition with a wall of 13 cm long $\times$ 5 cm height containing hole in the center.
- The cage is placed on animex activity monitor for counting the total locomotor activity of the animals under experimentation.
- An electronic system using four sets of photocells across the partition automatically counts movements through the partition. These photocells also note the time spent in the light and dark compartments.
- The animals are treated 30 min before the experiment with the test drugs or the vehicle intraperitoneally and are then observed for 10 min.

Advantages

1. The test is relatively simple with no painful stimuli to the animals.
2. This method helps in evaluating the potency of drug due to two compartment models, and also potency matches with clinical trials.

2.2.3 Social Interaction in Rats

Principle: Social interactions are a fundamental and adaptive component of the biology of numerous species. The main principle of this test is based on the free

choice by a subject mouse to spend time in any of three box's compartments during two experimental sessions. It includes indirect contact with one or two animals like rat or mice with which it is unfamiliar (Stack et al. 2010).

Procedure:

- The animals are placed in apparatus made up of Plexiglas chambers fitted with clean pine shaving.
- The size of the apparatus is adjusted in such a way that the adolescent and adult animals can freely move into it (30 cm × 20 cm × 20 cm for adolescents) and (45 cm × 30 cm × 20 cm for adults).
- The test apparatus is divided along the long axis into two equally sized compartments with Plexiglas partition that contained an aperture (7 cm × 5 cm for adolescents and 9 cm × 7 cm for adults) to allow movement of the animals between compartments.
- The hole is drilled in such a way that only one animal can be move through the aperture at a time.
- The animals are marked with any color on the back before the initiation of experiment in a holding cage for 30 min.
- For reducing the bias, the animals are exposed to pretest in which baseline level is measured by depriving them in a novel environment.
- After the training or pretesting period, all the animals are then individually placed into the testing chamber having a same age and sex test partner. Also, the animals should not be familiar with both the test apparatus and the experimental animal, i.e., with the paired animals already used for testing.
- Now record the behavior of the animal manually or by a video camera during the 10-min test session.

Advantages

1. This procedure is an useful one because animals are tested at two intervals, which reduce the experimental bias.
2. Mortality rate is zero because animals are socially interacted, and no harmful stimulus (like current) is used.

2.2.4 Open-Field Test

Principle: Open-field test is a simple and novel method which provides a unique opportunity to systematically assess general locomotor activity that is to screen anxiety-related behavior in rodents. In such procedure, the anxiety behavior of animals is directly measured timely without exposing them toward noise or other stimuli. In addition, higher the level of anxiety decreases the number of entries into the various boxes in the openfield apparatus.

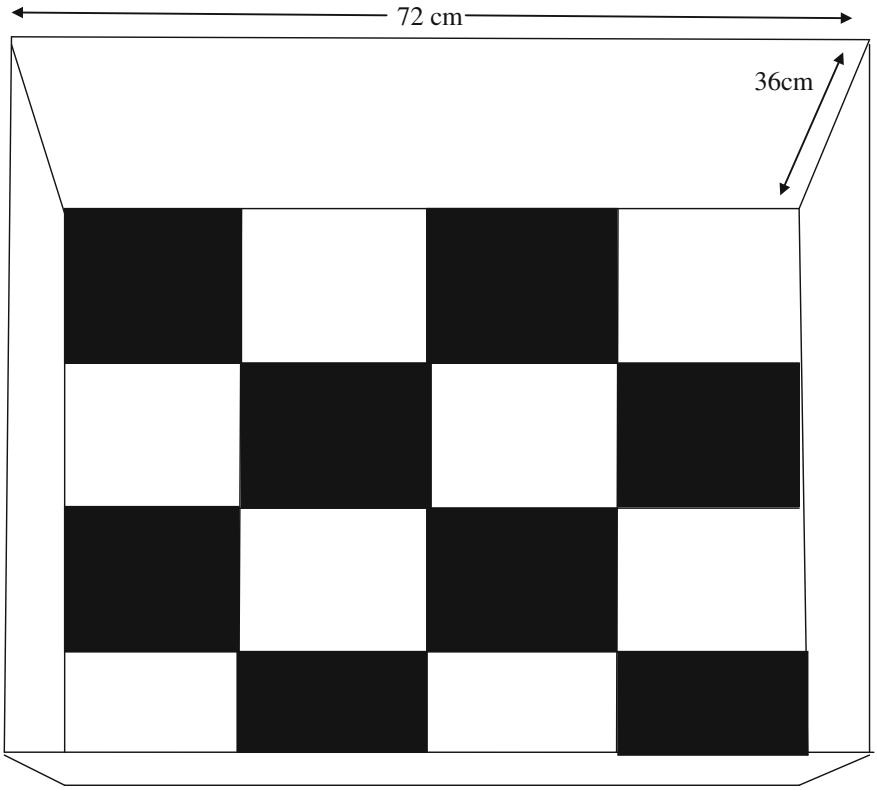

Fig. 4 Open field apparatus

Procedure:

- Open-field test is used to monitor spontaneous locomotor activity using wooden, rectangular, light brown or white black-colored open-field apparatus (100 × 100 × 40 cm) (Fig. 4).
- The floor of the apparatus is divided into 25 rectangular squares by pencil lines or with the help of marker. The experiment on the animal is performed in a room illuminated with 40 W white bulb located 150 cm above the test apparatus.
- After 2 h of first exposure of apparatus, the animal is placed in the center and number of squares cross/10 min by animal is recorded.
- Each crossing is considered only when the animal is fully moved with the four paws into the next box. Apparatus is cleaned properly after each trial and readings are taken.
- In addition to this, we can also record the horizontal units of activity, rearing behavior, defecation, and grooming activity. The maximum cut-off time provided to the animal is 5 min.

Advantages

1. It is helpful for measuring the physical motor ability of experimental animals.
2. Open-field technique is non-invasive and also do not require handling of animal's at each intervals.
3. The animal parameters are taken at stress free environment.
4. By using this test, we can check the number of behavior of animals like grooming, rearing and useful for evaluating the number of CNS disorders like Parkinson's, Huntington's, Alzheimer's, depression, and anxiety disease models.

2.2.5 Ultrasonic Vocalization (UV)

Principle: It is useful and reliable method for testing the anti-anxiety drugs in animals. In UV test, ultrasonic sound is used as indicator of the emotional and motivational status in animals. The ultrasonic vocalization directly indicates the behavioral state of animals and is suppressed by various drugs like benzodiazepines, serotonin (1A) receptor agonists, and selective serotonin reuptake inhibitors (SSRIs).

Procedure:

- The apparatus consists of Lucite box (30 × 30 × 50 cm) with two holes in which animals are trained (Knutson et al. 2002).
- Before the initiation of training session, the animals are habituated to the apparatus for 15 min. In this time, the number and duration of baseline free operant nose-pokes are recorded.
- For the smooth entry of animals, each hole is having a diameter of 3.1 cm and lies 5 cm above from on opposing walls.
- During the experimentation, the animals are placed into the apparatus and number of photo beam brooked is automatically counted in the computer along with frequency and duration of each nose-poke.
- Nose-pokes in the active hole are produced by playback of tape loop with system, recorded into a preamplifier and speaker fitted on the top of operant box.
- Animals are situated 50 cm away from the loudspeaker, and USV playback lasted as long as the animal continued to nose-poke in the active hole. However, playback is not elicited when the animals produce nose-pokes in the inactive hole.

Advantages

1. The model is useful to examine the subjective states of rats in addiction paradigms.
2. Ultrasonic vocalization is suitable method for rapid and repeated evaluation of newer anti-anxiety drugs (Knutson et al. 1999).

Disadvantages

1. It is a time-consuming procedure because in ultrasonic vocalization responding develops within five days, remains stable for at least 3 months and gives highly reproducible results later on.
2. All the animals' do not respond at same frequency of vocalization.

2.2.6 Stair Case Test (SCT)

Principle: Stair case test is used for the screening of anxiolytic and other psychopharmacological of drugs. The model is based on principle that the step—climbing is purported to reflect exploratory or locomotor activity, whereas rearing behavior is an index of anxiety state. In this the number of rearing and steps climbed latency are recorded in a 5 min period.

Procedure:

- The staircase test is carried out by the method. The apparatus is made of wood and consists of five identical steps 2.5 cm high, 10 cm wide, 7.5 cm deep surrounded by walls.
- The height of all the stairs is constant along the whole length of the staircase. On the second side of the stairs, a wooden box of dimensions (15 $\times$ 10 $\times$ 10 cm) is placed facing the staircase.
- The animal is gently placed on the floor of the box with its back to the staircase. After placing, immediately note down the number of steps climbed and rearing made for the time period of 5 min.
- The animal is considered climbed on a stem when all four paws are placed on the step.
- The number of steps climbed and the rearing responses are recorded for each animal. The apparatus is cleaned thoroughly before and after the recordings.

Advantages

1. It is a less time-consuming method because rearing index directly correlates to the anxiety state of animals.
2. The effectiveness of various anxiolytic drugs like benzodiazepines can be better evaluated by this model.
3. This model do not require food and water deprivation prior to training and also use natural stimuli.

2.2.7 Modified Hole Board Method

Principle: Modified hole board apparatus is used to explore the characteristic behavior of rodents in anxiety. The hole board setup is based on a previously

modified whole board that was designed to evaluate cognitive functions. In this, the animal when dipped the head into hole in a floor is considered as valid measure of its attraction toward novelty.

Procedure:

- The apparatus is made up of opaque gray PVC (60 × 20 × 2 cm) board in which 23 holes are drilled (1.5 × 0.5 cm) in three lines.
- All holes on the board are covered by movable lids made up of the same material. The hole board is placed in the middle of a PVC box (100 × 50 50 cm), which represents the central area of an open-field.
- By using marker or by drawing white lines the outer area is divided into 12 quadrates (20 × 16 cm). The size of the PVC box is enlarged by an additional compartment (50 × 50 × 50 cm), in which the experimental animal group are placed during the test period.
- Both the compartments, that is, group compartment and experimental compartment, are separated from each other by a transparent PVC partition perforated with 120 holes (1 cm in diameter).

Advantages

1. Simple method for measuring the response of an animal to an unfamiliar environment, with advantages that several behaviors can be readily observed and quantified in this test.
2. By the use of modified hole board apparatus, we can differentiate between low and high anxiety state of animals.
3. The method is cheap and also do not require any electric shock trials.
4. Modified hole board allows the animals to maintain the visual and olfactory contact to each other, and helpful in reducing stressful conditions of social isolation.

2.2.8 Novelty Suppressed Feeding (NSF)

Principle: The novelty suppressed feeding paradigm (NSF) is a conflict test. It elicits the competing motivations between the drive to eat and the fear of moving toward food pellets placed into the center of the box. Latency to begin eating is used as an index of anxiety-like behavior, because classical anxiolytic drugs decrease this measure.

Procedure:

- The test is performed in a apparatus consisting of box with dimensions 50 × 50 cm covered with bedding and illuminated by a 70 W lamp.
- During the first day, test animals are removed from its home cage and being placed in the corner of a novel test box, containing a single pellet of food (chow)

placed in the center. In this, the latency time to approach the chow and begin eating is recorded within a 5-min period.

- If the animal is anxious, it will avoid the food and display limited exploration of the test environment, whereas if the animals are less anxious they will approach the food quickly and begin eating.
- It has been found that chronic mild stress increases the latency time in the NSF test. This effect is reversed on administration of antidepressants. The antidepressant drugs show significant reduction in the latency to NSF.

Advantages

1. Decreased latency responses to the NSF in response to antidepressants have been associated with changes in hippocampal neurogenesis—a process that is thought to be important in the recovery from depression in humans.
2. The stress employed in these models is very mild relative to most other tests because simply the animal is placed alone into the box having a food pallet.

Ethical Statement

All institutional guidelines, national guidelines, state and local laws and regulations with professional standards for the care and use of laboratory animals should be followed. Studies involving animals must state that the institutional animal ethical committee has approved the protocol. For authors using experimental animals, a statement should be made that the animals' care is in accordance with institutional guidelines, and animals used have been treated humanely and with regard for the alleviation of suffering. Researchers should treat animals as sentient and must consider their proper care and use, and the avoidance or minimization of discomfort, distress, or pain as imperatives. Animal experiments should be designed only after due consideration of animal health. It should be ensured that all researchers who are using animals have received instruction in research methods and in the care, maintenance, and handling of the species being used. All the surgical procedures should be performed under appropriate anesthesia, and follow only those procedures which avoid infection and minimize pain during and after surgery.

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