EXPERIMENTAL MODEL FOR ANTIANXIETY ACYIVITY: A REVIEW

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Summary

Animal models can be used to obtain information about molecular mechanisms involved in anxiety that would be impossible in humans. In the field of anxiety research, there are two main categories of animal models: those that involve conditioned responses or unconditioned responses. Conditioned tests require extensive pre-test training and often use food/water deprivation or electric shock as an aversive stimulus. Unconditioned tests on the other hand do not require time-consuming pretest training as they measure un-learned, inherent anxiety. The present review article enlightens the various aspects of animal model of anxiety disorder, which may be use for research purpose.

Keywords: Anxiety, Conditioned test, Elevation, Elevated Plus Maze, Unconditioned Test

Introduction

Anxiety disorders are typically conceptualized as an interrelated pattern of responding that reflect two emotions: fear and anxiety [1]. Pathological anxiety is defined as fear without a relevant corresponding object or event. Animal models of anxiety are widely sought in an attempt to analyze pathological anxiety states with the assumption that some anxiety mechanisms are essential for survival and are a feature of all mammals [2].

While anxiety is believed to be a uniquely human trait, anxiety-like behaviors have been observed across many other species. An animal model allows investigators to test hypothesis under controlled conditions and using methods that would be difficult to manage in humans [3]. Unfortunately, many difficulties arise when modeling human psychiatric disorders, such as anxiety disorders, in another species. The major difficulty in modeling anxiety disorders in animals is that they are not capable of verbal communication, which is a critical component of diagnosing psychiatric disorders. It is difficult to identify analogous behaviors [4]. It is also difficult to distinguish between fear and anxiety. The behavioral and physiological responses in fear and anxiety are highly similar. The distinction between fear and anxiety lies in the concept that the former is a response to an actual threat while the latter is a response to a potential threat [5,6]. This definition is ambiguous in animals, so anxiety in animals can only be implied at best. Lastly, there are structural and functional differences between the nervous systems of animals and that of humans [4]. Therefore, the accuracy of the data obtained using animal models is dependent on the validity of the model.

Conditioned test

In the field of anxiety research, there are two main categories of animal models: those that involve conditioned responses or unconditioned responses [7]. Conditioned tests combine elements of learning and memory with aversive stimuli and require pre-test training paradigms. They measure a conditioned response, in other words, a specific response that is learned through association with an aversive stimulus. Examples of conditioned tests are fear-potentiated startle and Geller-Seifter conflict [8]. The required pre-test training associated with conditioned tests makes them more amenable to experimental manipulation than unconditioned tests [9].

Unconditioned tests

Unconditioned tests on the other hand do not require time-consuming pretest training as they measure un-learned, inherent anxiety. Unconditioned tests include the open field (OFB), light-dark transition and elevated plus maze (EPM) tests [3]. Unconditioned tests are believed to be more sensitive to stress compared to conditioned tests as the latter tend to use strong and often painful stressors such as foot shock. It is argued, that these stressors may suppress activity and cause complex changes in animal behaviors, making interpretation of results difficult [4]. Various experimental models for evaluating anxiolytic activity are discussed under subheads based on anxiogenic stimulus involved to induce anxiety in animals.

Elevation

The fear due to height (acrophobia) induces anxiety in the animals when placed on the elevated maze. The manifestation of anxiety and fear in the animals is exhibited by decrease in motor activity, which is measured by time spent by the animal in the open arms.

Elevated plus maze test

EPM is a simple and highly validated behavioral test for anxiety in rodents. The history of the EPM goes back to the 1950's when Montgomery observed that rats showed high levels of exploration and, therefore, preference for elevated enclosed alleys over elevated open alleys [10]. He inferred that since both alleys were novel and would, therefore, produce the same drive to explore, the rat's avoidance of the open arms was the result of fear response [7]. Based on Montgomery's findings, The maze consisted of two open arms set across from each other and two closed arms set at 90° to the open arms. The ability of the apparatus to measure anxiety-like behavior was established through the ability of anxiolytics and anxiogenics to affect the behavior within the apparatus [10]. Since its first description, the EPM test has been validated pharmacologically, physiologically and behaviorally, and has become one of the most widely used behavioral tests for anxiety-like behavior [10,11]. Montgomery's view that aversion to the open arms reflected fear or anxiety has been validated behaviorally as rats display more anxiety-related behaviors, including freezing and defecation, in the open arms compared to the closed arms. Physiologically, rats confined to the open arms show higher levels of plasma corticosterone than those confined to the closed arms [10-12]. As corticosterone is a stress hormone, its increased release strongly suggests that the open arms create an increased stress response in rats [10]. Finally, agents that increase anxiety levels (anxiogenics) decrease the amount of time spent in the open arms while agents that decrease anxiety (anxiolytics) predictably increase the amount of time spent in the open arms.

Elevated zero maze (EZM)

The maze comprises a black perspex annular platform (10.5 cm in diameter; 10 cm wide) elevated to 65 cm above the ground level, divided equally into four quadrants [13]. The two opposite quadrants are enclosed by a black perspex wall (27 cm high) on both the inner and outer edges of the platform, while the remaining two opposite quadrants are surrounded by perspex lip (1 cm high) which serves as a tactile guide to animals on these open areas. Rats are placed on one of the enclosed quadrants for a 5-minute test period. The maze is cleaned with 5% ethanol/water solution and dried between test sessions. Time spent in open quadrant, number of head dips over the edges of platform, numbers of stretch posture attained from closed to open arms are recorded.

Elevated T-maze (ETM)

The T-maze consists of three arms of equal dimension (50 x12 cm). One arm is enclosed by 40 cm high walls and disposed perpendicularly to the two opposing open arms [14]. The entire apparatus is elevated 50 cm above the floor. To avoid falls, the open arms are surrounded by a plexiglass rim 1 cm high. The animal is placed at the distal end of the enclosed arm of the ETM, facing the intersection of the arms, 30 min after treatment. The time taken by the rat to leave this arm with four paws is recorded (baseline latency). The same measurement is repeated in two subsequent trials (avoidance 1 and 2) at 30 sec intervals. Following avoidance training, rats are placed at the end of the right arm of the maze and the latency to leave this arm with the four paws is recorded for three consecutive time interval (escape 1, 2 and 3), each of 30 sec.

Novel environment

Exposure to novel environment induces approach-avoidance behavior in animals.

Open field behavior (OFB)

The apparatus used for this purpose consists of a white circular plexiglass floor (40 cm in diameter) surrounded by a grey PVC wall (30 cm in height) [15]. The floor is divided into one central and six peripheral parts of equal surface. A 100 W white bulb placed 70 cm above the open field provides a 500-lx illumination at floor level. At the beginning of the test, the mice are placed in the peripheral part of the open field and video recorded for 5 min. Following neurobehavioral parameters are recorded:

- Latency before leaving the initial part
- Total number of entries into peripheral and central parts
- Rearing and defecation
- Stretched attend postures

Hole board test

The apparatus consists of a grey perspex panel (40x40 cm, 2.2 cm thick) with 16 equidistant holes (3 cm diameter) in the floor [4,16]. Photocells below the surface of the holes provide the measure of the number of head dip. The board is positioned 15 cm above the table and is divided into 9 squares of 10x10 cm with black water-resistant marker. Each animal is individually placed in the centre of the board (facing away from the observer) and following parameters are noted during 5 min test period: (a) the latency of first head dips, (b) number and duration of head dips and number of rearings, and (c) spontaneous movements (number of squares crossed with all four paws).

Social interaction in rats

In an unfamiliar and brightly lit environment, the normal social interaction of rats (sniffing, nipping, grooming) is suppressed. Anxiolytics counteract this suppression. The apparatus used for the detection of the changes in social behavior and exploratory behavior consists of a perspex open topped box (51x51x20 cm) with 17x17 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 into the box (with 60 W bright illumination 17 cm above) and their behavior is observed over a 10 min period by remote video recording. Social interaction between the animals is determined by noting the time period of sniffing of the partner, crawling under or climbing over the partner, genital investigation of the partner, and following the partner. Exploratory motion is measured as the number of crossing of the lines marked on the floor on the test box. Six pairs are used for each dose. The anxiolytic activity is determined by comparing the values of treated partners with the data from pairs of untreated animals using single factor analysis of variance [17].

Suppression of feeding by novelty

The apparatus consists of a wooden box (60x60x35 cm) with a solid floor. The floor is covered with a 2 cm wooden chip layer, and 15 laboratory chow pellets are evenly placed on the floor. A similar arrangement is made in home cage rats. The rats are not given any feed 24 h prior to testing, but are provided with drinking water. The rats are placed individually in the test chamber and the latency to begin feeding is recorded. If the rats do not eat within 300 sec, the test is terminated and a latency score of 300 sec is recorded [18].

Staircase test

The staircase test is used for evaluating anxiolytic activity by purporting step-climbing to reflect exploratory or locomotor activity, while rearing behavior is an index of anxiety state [17]. For experiments with mice, the staircase is composed of the five identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. The internal height of the wall is constant along the whole length of the staircase. In this test, each animal is used only once. Twelve mice each are used for the untreated control group, drug group, and for the group receiving standard. The drug or the standard is administered orally 1 h or 30 min prior to 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 minute period. A step is considered to be climbed only if the mouse has placed all four paws on the step. In order to simplify the observation, the number of steps descended is not taken into account. After each test, the box is cleaned in order to eliminate any olfactory cues which might modify the behavior of the next animal. In this experimental model, average number of steps and rearing of control group is taken as 100%, and the values of treated animals are expressed as percentage of the control.

Light/dark model

Exploration of mice or rats is inhibited by bright illumination, which is highly aversive for rodents. In light and dark model, animals are placed on the brightly lit side of a two compartment chamber and the number of crossings between the light and dark sites is recorded [17]. Anxiolytics produce a dose-dependent increase in crossings. The apparatus consists of a cage which is one third darkened with a cover and separated with a wall from the otherwise brightly illuminated area. A round hole (diameter 13 cm) allows the rat to pass from the illuminated to the darkened compartment. The cage is placed on an Animex®–activity counter. The animals are treated orally with the test compound 30 min before the session. A group of 6-8 animals is used for each dose. At the start of the test, the rat is placed in the middle of the illuminated part of the cage. The number of crossings is registered during 10 min. The anxiolytic activity is screened by comparing the average number of crossings in the treated groups with the saline treated control.

Mirrored chamber test

It has been observed that animal species exhibit approach-avoidance response upon placing of a mirror within their environment. The parameters, i.e., latency to enter, and total time spent in the mirrored chamber can be used to evaluate anxiolytic drugs.

The apparatus consists of a mirrored cube (30 cm on a side) open on one side that is placed inside a square wooden box (40x40x30.5 cm). The mirrored cube consists of five pieces of mirrored glass. The mirrors used are mirrored on one surface only (back surface being painted dark brown). The three mirrored side panes, a top pane, and the floor pane face the interior of the cube. The mirrored cube is placed in the centre of the wooden container to form a 5 cm corridor which completely surrounds the mirror chamber. A mirror is also placed on the container wall so that it faces the single open side of the mirrored chamber. The other three walls of the container are painted dark brown.
Thirty min after the administration of the test drug or standard, the animals are placed individually in the chamber of mirrors at a fixed corner. During a 5-min test period, the following parameters are noted: (a) latency to enter the chamber, i.e., the time spent, in sec, for the first entry into the chamber of mirrors, (b) number of entries in mirrored chamber, and (c) the time spent with each entry is calculated by dividing the total time spent with number of entries[19,20].

Physical discomfort

Four plate test in mice

The four plate test is used for evaluating anxiolytic activity by delivering the shocks to reflect locomotor activity [17]. The test box has the shape of a rectangle (25x10x16 cm). The floor is covered with four identical rectangular metal plates (8x11 cm) separated from one another by a gap of 4 mm. The plates are connected to a source of continuous current which applies to two adjacent plates a mild electrical shock of 0.35 mA for 0.5 sec. Adult male Swiss albino mice are randomly divided into different groups. Thirty minute before the test, the animals are administered the test drug or the vehicle.
At the beginning of the test, the mouse is gently dropped onto a plate and is allowed to explore the enclosure for 15 sec. After this, every time the animal crosses from one plate to another the experimenter electrifies the whole floor for 0.5 sec which evokes a clear flight reaction in the mouse. 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 minute is taken as the parameter for evaluating anxiolytic activity.

Foot shock-induced freezing behavior is rats

In this test, anxiolytic activity is determined by freezing behavior. The animals receive a single test compound or the vehicle 30 min prior to being placed in a standard conditioning chamber (e.g. Coulborn Instrument) for a 6.5 min session. Two min after the start of the session, a scrambled foot-shock (0.5 mA, 0.5 sec) is delivered through the grid floor of the chamber. Using an assembly of push buttons interfaced with a computer, an observer monitors the amount of time each animal spends engaged in the following mutually exclusive types of behavior.

- Freezing: immobility with rigid body posture.
- Sedated posture: sitting or sleeping.
- Small exploratory movements: movements involving the torso or front paw only, vertical movements of the head or sniffing.
- Locomotion: activity involving hind jaws, grooming or rearing.

Frequency of rearings is also counted. All types of behavior are monitored for the entire 6.5 min session. Evaluation of the antianxiety effect can be done on the basis of duration of foot-shock induced freezing after administration of test compound and control [17].

Distress vocalization in rat pups

It has been observed that when rat pups are held by tail, they emit ultrasound. This parameter, i.e., number of sounds produced by rat pups can be used to evaluate anxiolytic drugs. The pups are tested at 9-12 days of age. In the morning, all pups are subjected to handling stress and the magnitude of their ultrasound emission is observed. The stress consists of holding the pup by the base of the tail between forefinger and thumb of the experimenter, and then suspending it 5 cm above the bench for 30 sec. A control recording (30 sec) is taken when the pup is held gently, whereby the pups emit only a few ultrasounds. Responses when held by the tail are more than 10 times higher. The entire hand-tail holding procedure is immediately repeated. Ultrasounds are recorded with suitable detectors with 42 KHz as the center of a 10 KHz recording range. The output of the detectors is fed into pen recorders. The total number of ultrasonic cries in the two sessions of hand holding and the two

sessions of tail holding are calculated. These are used as the control activity of each pup. Any pup producing less than 50 ultrasounds when held by the tail is excluded from the drug study. Three to four hours after the first test, the pups are randomly allocated to several equally sized groups, weighed, marked, treated with the vehicle or drug, and placed back in the home cage. Thirty min after treatment, each pup is subjected to the same handling stress which is used in the morning session, and the total number of sounds produced is calculated in the same way. The antianxiety effect can be evaluated by comparing the total number of sounds produced by the treated group and control.

Anticipatory anxiety in mice

It has been observed that when group-housed mice are removed one by one from their home cage, the last mice removed have always higher rectal temperature than those removed first. This parameter, i.e., anticipatory fear for an aversive event (handling causes stress induced hyperthermia) can be used to screen anxiolytic activity.
In this experiment, mice are housed at constant room temperature and relative humidity for at least seven days in Makrolon cages to adapt to the environment. Test drugs or standard (diazepam) drug are administered orally in various doses to the group of 18 mice prior to the test. Thirty min later, first mouse is removed from the cage and the rectal temperature registered by inserting a silicone lubricated thermistor probe (2 mm diameter) upto 2.5 cm into the rectum. The average temperature of 3 mice is taken as basal value. Mice number 4 through 15 are simply removed and again returned to the cage, and thereafter body temperature is determined in the remaining three animals. The difference of the mean value of these mice and the basal value is calculated as increase in the body temperature, i.e., anticipatory fear [17].

Conditioned conflict procedure

Vogel's conflict test

To check the antianxiety activity, simple and reliable conflict procedure is used. Thirty rats are administered shocks while licking water. The apparatus is a clear plexiglas box (38x38 cm) with a black plexiglas compartment (10x10.5 cm) attached to one wall and an opening from the large box to the small compartment. 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 is extended into the box at a height of 3 cm above the grid. Rats lick in bursts with a relatively 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 by switching the connections to the drinking tube and grids from the drinkometer to a shocker which applies an unscrambled shock between the drinking tube and grid floor. The rat is placed in the apparatus and allowed to find the drinking tube and to complete 20 licks before shock (available at the tube for 2 sec) is applied. The rat controls the shock duration by withdrawing from the tube. A 3 min timer is automatically started after the termination of the first shock. During 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. The number of shocks received after treatment is compared with untreated animals.

Geller type conflict test

Animals are subjected to food deprivation in order to induce hunger [21]. They are trained under MULT FR20/FR20-punishment schedule of food reinforcement, using the apparatus for the Geller type conflict test (GT-8510, GT-8005 and GT-7715). The schedule consists of four pairs of an alternating safe period; the mouse's lever pressing is reinforced by food pellets at FR20 without electric shock. During the alarm period, which is indicated by a warning stimulus (tonic signal: 80Hz, 90 dB), every 20th lever press is punished using an electric shock (50-90 V, ca 0.3mA, 50 Hz AC, duration 0.3 sec). The response rate of the animal is recorded during the safe as well as alarm period.

Social separation

Social separation stress results in vocalizations and nociceptive responses in animals, and is sensitive to the anxiolytic drugs.

Chick social separation-stress

In this procedure, a chick is placed into the observation chamber in isolation for a 3 min test session (Sufka et al., 2001). To index stress-induced analgesia, a 50 µl injection of 0.10% formalin is administered into the plantar region of the Chick's foot immediately before placement into the chamber. The following observations are recorded [22].

- Foot lift frequency and foot lift duration in response to formalin
- To index sedation
  - Ventral recumbent latency that resembles a sleep-like posture. A composite pain score (CPS) is derived from the following formula. CPS = 4 (z-score foot lift) + [z-score (duration/total number of lifts)].
  - Number of vocalization to index separation-stress.

Exposure to predator/predator's odor

Cat odor exposure

The paradigm is based on defensive behavior displayed by rodents when confronted to a predator or to its odor.

Mice are divided into three groups, i.e., control (no odor), neutral door (modeling clay) and predator odor (cat feces). Cat feces are obtained from a 2 year old male domestic cat, collected out doors quickly after defecation. The neutral odor is provided by a 2 cm diameter ball of blue modeling clay of the same volume as cat feces. The mice are individually brought to the testing room dimly lit with a 40-W red bulb (15-lx on the testing location). After the removal of the grid, and depending on the group, an odorant stimulus is placed on the saw dust, at the opposite location of the food compartment. The same procedure is followed for the control group except that no stimulus is given. In order to avoid any disruption between odors, the mice from the control group are always tested first followed by those from the neutral odor group and the predator odor group. The grid is then replaced and the behavior of each mouse is recorded from the side during 5 min [23,24]. The parameters recorded are discussed below.

(a) The time spent at the opposite location of the odorant stimulus (b) The number of entries into the tow parts of the cage (i.e. under the food compartment or stimulus location.)

(c) The number of contacts with the odorant stimulus.
(d) The number of stretch attends posture.
(e) The number of burrows in odorant stimulus.

The cat odor can also be obtained by rubbing a damp cloth (20x20 cm) against the fur of male laboratory housed domestic cats for 5 min [25]. Clothes with cat odor are kept in sealed plastic bags. Each cloth is used for four exposures only.

Mouse defense test battery

In this assay, mice are placed in a test alley and approached with an anesthetized rat, a natural predator of mice that triggers unconditioned aversive reactions [25]. A series of typical behaviors is assessed including flight, risk assessment (repeated episodes of extending and withdrawing body in the direction of threat) and defensive threat and attack response.

Anxiogenic agents

Antagonism of discrimination stimuli produced by anxiogenic drugs

It has been seen that most of anxiolytics block the discriminative effect of pentylenetetrazole (PTZ), whereas anticonvulsants without anxiolytic effects do not [26].

A sub-convulsive dose (20 mg/kg, i.p.) of PTZ is used as an anxiogenic stimulus in rats. The basic method involves training of rats to press an appropriate lever for food in the presence of an injection of PTZ. Responses on second lever are also recorded. A rat is considered trained when it can reliably press the lever appropriate to a PTZ versus saline injection. Treatment with anxiolytic drug blocks this discriminative effect of PTZ.

β-Carboline-induced behavioural syndrome in monkeys

Ethyl ester of β-carboline-3-carboxylate (β-CCE) possess a high affinity for BZD receptor binding site, and, therefore, causes a behavioural syndrome, which is blocked by diazepam [27]. The procedure involves chaired monkeys with in-dwelling intravenous catheters. β-CCE is administered i.v., and type of behavior includes marked head and body turning, and distress vocalization. Plasma samples show increases in cortisol, epinephrine, and norepinephrine. Drugs with antianxiety activity are considered to block these behavioural changes. This model is time consuming. Moreover, rhesus monkeys are expensive and difficult to obtain. These disadvantages associated with this model limit its use for preliminary screening of anxiolytic drugs. Therefore, this procedure fits best as a 'tertiary' evaluation, after there is sufficient evidence of anxiolytic potential from other preliminary measures.

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