

## **Models to Evaluate Anti-Anxiety Effect**

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### **Review Article**

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#### **ABSTRACT**

Nowadays various models are available to evaluate anxiolytics effect of a new entity or even for comparative studies with the standard drug. Animal models are very useful to understand the pathogenesis of the disease and also helpful in finding the treatment of the particular disease. As per the ethics, a new compound or drug believes to have possible pharmacological effect should be tested on animals before tested on humans which have similar physiology than humans. Anxiolytics are the agent which are used to elevate anxiety effect produced due to any cause. Whenever a new drug believes to have a possible pharmacological response, an animal model is developed to verify the effect of the drug which study is also known as a preclinical study. First, rats were used as an animal model for evaluation of anti-anxiety drug but later on the various strain of mice were added to an animal model for evaluation of anxiolytics because of better genetic possibilities than rats. In this review article, we have discussed the most commonly used four animal models used to evaluate the anti-anxiety effect. The various parameter will be undertaken for the better and precise evaluation of anxiolytics. Behavioral studies can also be conducted with the help of some animal models used to evaluate the anti-anxiety effect.

### **INTRODUCTION**

#### **Anxiety**

Anxiety is a medical state related to our psychological as well as physiological behavior having numerous characters like cognitive, emotional, behavioral and somatic. The term anxiety actually came from a Latin word "Ango" which means "to vex or torment" may be in the absence or presence of any psychological stress. It can also create a feeling of worry, feeling of fear, feeling of uneasiness. Sometimes, it is considered a normal reaction for a stressor.

GABA-A receptor system functionally associated with regulation of anxiety. Evidences which are available point out the major role of  $\alpha$ -2 subunit of GABA-A in modulation of anxiety and as a result of present studies it is suggested that  $\alpha$ -3 subunit of GABA-A receptor could be associate with anxiety [1].

Recent data (2017) of anxiety shows that 284 Million people are affected by this disorder worldwide which includes 63% females [2].

There are 4 types of anxiety: Generalized anxiety disorder, Specific phobias, Social anxiety, and Panic disorder. There are two other conditions where anxiety disorder also present i.e., Obsessive-compulsive disorder (OCD) and Post-traumatic stress disorder (PTSD).

In case of anxiety, adrenaline is released by the nervous system when our brain sends the message in the case when we feel some danger or think that something dangerous is about to occur as a result, we feel alert and energetic and prepare us to attack or to move to safety. Mostly, the effect of anxiety is lived for the short time period when we encounter something unpleasant or outside of our comfort level but when the symptoms are severe and remains for longer duration and affects negatively on the personality, ability to work and socially which is considered as a problem [3].

There are several factors which affect the person need to be considered are:

- The degree of distress caused due to the anxiety symptoms
- The level of effectiveness of the anxiety symptoms have on a person's ability to study or work and to socialize and manage daily task

- The context in which anxiety occurs

## DIFFERENT MODEL FOR EVALUATION OF ANTI-ANXIETY DRUGS

There are various models are present nowadays to evaluate any new drug moiety or in combination and to compare with the standard drug for an anxiolytic effect.

1. Elevated Plus Maze
2. The Light and Dark Box
3. Marble-Burying Behavior
4. The Hole-Board Test

### Elevated plus maze

This model is used to evaluate behavior related to anxiety disorder in rodents. This is the common and most simplified model to evaluate the anti-anxiety effect. In early time, an elevated Y-shaped apparatus was offered by Montgomery in 1955 to evaluate the anxiolytic effect but later on, it was modified by Handley and Mithani in 1984 [4] and Pellow et al. in 1985 into an elevated plus maze with two open and two enclosed arms which were arranged to form a plus shape maze. Afterwards, the same apparatus was offered by Lister in 1987 for mice [5]. This model was investigated to evaluate fear-induced behavior i.e., anxiety. The Elevated-Plus Maze helps in rapid screening of drugs which modulate anxiety or mouse genotype such as 5-HT 1A KO, CCK 2 KO without involvement of complex schedules or training. It has an advantage over other models which uses food or water deprivation or shock administration to assess anxiety [6].

A day before experiment animals should be placed in each arm of the Elevated-Plus maze so that animals get familiar with the apparatus and will not take time to respond while actual activity will perform.

Dimensions are shown in the figure for the Elevated-Plus Maze apparatus. As shown in the figure, it consists a plus shape maze elevated above the ground with four arms i.e., two closed arms with an open roof and two open arms positioned oppositely. Measurement of the apparatus can be changed according to the type of study and to get the precise result. There are different measures for different species i.e., For the rats, maze should be approx. 70 cm above the ground with the length and breadth of the arm is approx. 45 cm and 10 cm respectively and for the mice, maze should be approx. 30 cm above the ground with the length and breadth of the arm is approx. 30 cm and 5 cm respectively [7]. It also consists of a centre point where the animal will be placed during the experiment. A video camera also mounted above the maze for the recording of the subject's movement and analysed by a video tracking system. As rodents prefer to live in dark places and avoid light or bright places to live plus, they also have an anxiety effect with height. Therefore, time spent in the open or closed arm is calculated to measure anxiety-like behavior or the anti-anxiety effect of a drug. 70% ethanol should be used to clean the maze after each and every trail and at the end of the day it should be cleaned with 10% bleach [8].

After administration of drug experiment should be started according to dose and their peak plasma level. The animal should be kept in the center with their head facing towards the open arm and the animal will start moving in different arms as per their condition. The behaviour which is typically recorded is the time spent and entries made on the closed and open arms. In this task, behaviour reflects a conflict between in their living preference for protected areas i.e., closed arms and their innate motivation to explore novel environments i.e., open arms.

The number of rears, head dips, faecal boli, freezing or stretched-attend postures are the other ethological measures that can be observed in this model.

In Elevated Plus Maze, there is no such evidence of age-related restriction in behavioural testing. There is various data available which support that all age group rodents can be used to evaluate anti-anxiety activity of a drug [9]. But later on behavioral study was conducted which introduce that the animals having age less than 3 months experienced less anxiety on elevated plus maze and dim illuminating effect in elevated plus maze also encourage the exploration of open sides of maze [10].

Anxiolytics would be expected to increase the proportion of entries and time spent in open arms.

### The light and dark box

This is another model used to evaluate the anxiolytic effect in rodents. The benzodiazepine is the only drug that shows the consistent result in this model. It has an advantage over other paradigms that it does not require prior training and easy to use and quick process [11]. The light/dark model was introduced by Grawley and was validated by Godwin in 1980 and Costal in 1989 by variation of illumination within the box and uses a different type of strain of mice [12]. In a study, an experiment was conducted on the different age group to check the whether the light and dark test reflect anxiety in each age group and it was found that adolescent rats were emerging in light more quickly than adult aged rats. It was suggested that locomotor activity is more associated with anxiety-like behavior in adolescent rats than in adult rats. In the number of light entries, no age difference was detected in between different age groups but it was found that adults made more poke in a light box than adolescents [13].

Another study was done to evaluate anxiety-like behavior in different strains of mice such as CD-1, BALB, DBA, B 6 and their

behavior was studied for 15 minutes for each mouse. As a result, B 6 and DBA are the strains prefer light and dark box over open field test while BALB strain highly avoids the light and the dark box [14]. Later on, another study shows mice were more active in terms of undirected exploration and locomotion in their behavior and this study also indicates impairment in cognitive behavior if the study is done during the light phase as paralleled by increased avoidance behavior which is an indication of anxiety disorder [15]. Illumination also has an effect on behavior of mice which was studied and it is found a significant difference between normal and albino mice that they preferred to remain in dark box when the ambient condition in the light box was 1000 lux while no preference was shown in any compartment when it was 0 lux [16]. Another study was done on both rats and mice and it was figured out that both rat and mice avoid the illuminating side as illumination potentiate the acoustic startle reflex which is count as a measure of anxiety [17].

Further studies show that cholinergic mechanism may be involved in restraint stress-induced neurobehavioral alteration. The time spent in the light chamber of the light and dark box was also reduced whereas pre-treatment with atropine before the restraint procedure reverse all changes induces by the restraint stress [18].

Its construction is very simple and is made up of wood having two chambers one bigger (Painted White) and one smaller (Painted Black) which is separated by a thin wall. A tiny gate is also available in a corner of the wall to allow the animal to pass through one box to another box. A light source is also placed in the white chamber and the upper side of the apparatus is covered with a wooden plate.

Now, place the mice and allow the animal to travel from one chamber to another chamber. The total entries on each side and total time spent on each side are counted for a specific time i.e., 5-10 minutes is counted. An entry will be considered if all four paws of the animal must be placed into the opposite chamber. Now, administer the drug and again place the mice and perform the experiment as per the doses and peak plasma level. Scaling at the bottom of light and dark chamber can be used for an exact measuring of distance travelled. The movement of mice in light and dark chamber and total time spent is recorded. This light and dark box model can be used to evaluate both the anxiogenic-like and anxiolytic-like activity of drug [19].

Anxiolytics would be expected to increase the percentage of time spent in the light box.

### **Marble-burying behavior**

This is another model to evaluate the anti-anxiety effect or Obsessive-Compulsive Disorder behavior i.e., This model is able to detect phenotypes related to anxiety disorder and Obsessive-compulsive disorders. It is also helpful in pharmacological and behavior testing. In mice, the burying of the marble is a natural defence mechanism that occurs under anxiety or condition of stress. Neophobia generally found in rodents which means fear from new or strange objects. When they meet with new or strange or noxious or harmful objects, they start showing some specific type of behavior such as burrowing, digging, rearing, burying, grooming and hoarding. It means when the animal (rodents) are put in the cage with the marble they will start to bury the marble. According to studies it is seen that mice were the most preferably used in marble-burying behavior model. There is the various strain of rats such as Brattleboro, Wistar Albino, Nile Grass, Sprague Dowley, Wistar Kyoto, Lewis Rat and mice such as Swiss Albino, CD 1, C 57B 1/6J, BALB/c, Swiss Webster Albino and NMRI mice were used and found significant variation. Lighting does have some significant effect as high-intensity light triggers off stress. So, the light and dark box must be enlightened with 60-150 lux. Handling and method of administration of the drug may vary the result. The temperature must be 23-25°C i.e., room temperature as variation in temperature may also vary the result. Apparatus must be cleaned with 10-70% ethanol and dried completely [20].

Standard polycarbonate cages (26 cmx 48 cmx 20 cm) are used with the fitted filter-top covers. Unscented bedding material is added to the cage to a depth of 5 cm [21]. 24 Marbles are evenly distributing across the bedding and leave the animal for 30 minutes testing period. The same procedure will follow after the administration of the drug. Each animal is returned to its home cage after 30 minutes and all the marble buried 2/3<sup>rd</sup> or more will be count where less than 2/3<sup>rd</sup> buried marbles will not be count.

Recent studies show that GABA is involved in regulating compulsive behaviors along with serotonin. Thus, a marble burying behavior model may prove more useful for pharmacological drug tests of impulsivity or attention deficit because of the involvement of GABA and serotonin in both the disorders [22]. This burying behavior of rodents was first introduced by Pinel and Treit 1978. It shows that the burying of marble cannot be correlated with exploratory activity or any measure of anxiety-related response as it might be related to an animal's digging or burying activity which does not support anxiety disorder. As marble burying behavior is genetically regulated and it cannot be related to anxiety measure in other assays [23]. It also shows that the testing is independent of sex i.e., male and female both show the same type of behavior and result i.e., the number of marbles buried but most marbles were buried when they were evenly spaced. Some drugs at different doses increased and reduced the burying of marbles and also have no effect on burying on marble (e.g. diazepam at 0.1mg/kg, 0.25mg/kg and 1.0-5.0mg/kg increased, no effect and has reduced the burying of marbles respectively [24].

In a recent study, it is figured out that repeated exposure to the marbles does not cause habituation in animals suggesting that it is not related to fear because marble is non-reactive and they cannot cause harm to animals hence this 'frustrated' investigation leads to the compulsive burying. Originally, marble burying was concluded as anxiety-related behavior but later on, it was found that drug acting as serotonin reuptake inhibitors reducing the marble burying behavior which is related to obsessive-compulsive disorder. This is still a matter of debate that whether marble burying behavior measures anxiety or compulsivity [25]. Another study encourages that marble burying behavior model can also be used to evaluate drugs used in obsessive-compulsive disorder

as cannabidiol reduced the repetitive behavior and containing anti-compulsive properties [26]. As per literature, anxiety is a relevant dimension of obsessive-compulsive disorder which may impact the other patient's characteristics [27]. Therefore, it is studied that marble burying may or may not reflect both neophobic/defensive (anxiety-like) behavior and inherent (compulsive-like) burying behavior [28]. In marble burying behavior, marble burying is used as an index of anxiety disorder. However, it has been argued that the marble burying behavior model is more ethologically relevant to obsessive-compulsive disorder than any other type of anxiety disorder [29].

Another study has shown that the physical properties of bedding material and the number of marbles used in the cage significantly affect the marble burying behavior. The texture and density of bedding material are responsible for the burying of marbles. As in the observation, it was observed that more marbles were buried by mice in low density and soft bedding than high density and hard bedding. Spontaneous activity of mice was also affected by bedding material by both quantitatively and qualitatively. When mice were exposed to fresh bedding then it shows high activity [30].

Anxiolytics would be expected to decrease the burying of marble.

### **Hole-board test**

This is another model used to evaluate the anti-anxiety effect. There were various flaws in the Open-field Model so to overcome it was first introduced by Boissier and Simon in 1962 [31] and then it was developed by File and Wardill 1975a, b; File, 1981, 1985. As head dipping is a point of measurement in the hole-board apparatus which was considered as a Neophilic (Subject's attraction towards novelty) condition but the study suggested that when the animal was exposed first time to the apparatus, the animals have shown neophobic characteristic. Thus, any behavior observed in a novel environment can be influenced by both neophilia and neophobia [32]. It is also able to measure multiple behaviors that is why it is the most popular model to evaluate the anxiolytic effect. Various exploratory behavior can be evaluated in the hole board test such as head dipping and rearing. It was found that treatment with anxiogenic and anxiolytic agents affected head dipping behavior. Thus, this model can be useful in the evaluation of various changes in the emotional state of mice [33].

Traditionally, 16 holes were evenly distributed on the floor of a box (40 x 40 x 25 cm). Infrared beam falling on photocells are available below the floor. The animal was placed in the box and records its locomotion (movement from one place to another place) and other behavior such as head dipping (when animal dip his head in the hole until its ears are level with the floor and break the infrared beam and rearing is when the animal stands on its hind legs. The locomotion activity is used to measure by using Actophotometer as the movement of an animal interrupts a beam of infrared light falling on a photocell, at which the count is recorded and displayed digitally [34].

The modified whole board apparatus can also be used to assess multiple dimensions of various unconditioned behavior mainly in mice and rats. It includes traditional hole board apparatus and open field tests which overcomes the various disadvantages of test battery such as reducing the number of animals used, reducing time-effect and cost. The main advantage is that animals do not need to be deprived of food to increase the motivation to solve the task. This apparatus is validated for both mice and rats.

Various behavior test can be performed with the help of a modified hole board apparatus are:

- a.** Risk assessment
- b.** Avoidance behavior
- c.** Arousal
- d.** Exploration
- e.** Habituation
- f.** Cognitive
- g.** Social affinity
- h.** Locomotor activity
- i.** Social stress experiment
- j.** Novel object recognition test

It consists of an experimental box (100 x 50 x 50 cm) which was separated from an additional compartment (50 x 50 x 50 cm) by a transparent partition. In the middle of the box, a board is placed consist of some cylinders according to the test protocol. A light was also placed in the middle of the box referring to the red zone i.e., unprotected area. Two strains of mice were used in the experiment, as a result, BALB/cJ mice have shown the decreasing habituation when test under the red light condition and in normal light condition while 129P 3/J has not shown any change under any condition [35]. However, testing under white light conditions may induce cognitive disruption and behavioral inhibition. In this model, it is possible to analyze the behavior of rodents and thus, it characterizes the animal model which mimics a psychopathological condition plus it allows to reduce the time needed and the number of animals used to perform a behavioral phenotyping [36].

Another study has shown that the hole board apparatus can be used to measure preference and avoidance response to biologically

relevant odors. Various samples were placed in holes such as female urine sample, carnivore urine sample, omnivore urine sample and herbivore urine sample. As per observation, hole containing the carnivore sample was totally avoided while a hole containing female urine sample were preferred. When rats were treated with buspirone (an anxiolytic drug) avoidance of hole containing urine sample was completely abolished which indicates a specific anxiolytic action induced by the carnivore urine sample [37]. However, this model is inappropriate for the evaluation of the compound containing high sedation effects. Another study encourages the latency of head dipping when methanolic extract of *Holoptelea integrifolia* leaves administered orally, which indicates anxiolytic enhance the latency of head dipping of rodents when tested in hole board apparatus [38]. In the previous study, it is reported that rearing behavior is increased by dopaminergic transmission via inhibition of D<sub>3</sub> receptors mainly found in limbic and striatum system. The exploratory activity also has been found to increase by catecholamines and serotonin by inhibition of monoamine oxidase which degrades them in the brain. Therefore, exploratory activity was enhanced by anxiolytic agents in hole board paradigm [39].

Nowadays, the use of transgenic animals has been encouraged due to specific and optimum outcomes. Hence, transgenic mice can also be used to evaluate behavioral effects of altering a specific type of neurochemical receptors i.e.,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxasole-propionate, N-methyl-D-aspartate, Dopamine, Serotonin and Metabotropic glutamate [40].

Anxiolytics would be expected to increase these behaviors such as locomotory, head dipping, and rearing. The animal is more anxious if the animal doesn't show these behaviors.

## COMPARATIVE STUDY OF ANTI-ANXIETY MODELS

| Models                               | Invented by         | Animals used        | Response to be observed                                      | Inference of the observation   | Clinically tested available drugs   |
|--------------------------------------|---------------------|---------------------|--|--|---|
| <b>Elevated Plus Maze</b>            | Handley and Mithani | Mice, Rats, Gerbils | Change in entries and time spend in open arm                 | If increase: drug shows its anxiolytic effect &<br>If decrease: drug does not have anxiolytic effect | Benzodiazepines[41], Barbiturates[42], Prazosin[43], Clonidine[44], 5-HT <sub>1A</sub> receptor agonist[45], Ethanol[46], Ondansetron[47] |
| <b>The Light and Dark Box</b>        | Grawley and Godwin  | Mice and Rats       | Change in entries and time spent in light box                | If increase: drug shows its anxiolytic effect &<br>If decrease: drug does not have anxiolytic effect | Paroxetine and Buspirone[48], Dothiepin[49], Moclobemide[50], Benzodiazepines [51]  |
| <b>Marble Burying Behavior Model</b> | Pinel and Treit     | Mice and Rats       | Change in the number of marbles buried                       | If increase: drug does not have anxiolytic effect &<br>If decrease: drug shows its anxiolytic effect | Diazepam and Pentobarbital[52] Nitrous Oxide[53], Progesterone[54], Selective Serotonin Reuptake Inhibitor[55]                            |
| <b>The Hole Board Model</b>          | Boissier and Simon  | Mice and Rats       | Change in locomotory, head dipping and head rearing behavior | If increase: drug shows its anxiolytic effect &<br>If decrease: drug does not have anxiolytic effect | Paroxetine[56], Chronic Tiagabine[57], Benzodiazepines[58]  |

## CONCLUSION

Nowadays, everyone has a topic of becoming anxious. Each and every person from student age to older age everyone is affected by tension, fear and other unpleasant factors. Anxiety is common nowadays but it is counted as a disorder when its symptoms remain last long. Generally, symptoms of anxiety are short-lived but when it starts affecting the functioning systems mainly cognitive, physical and behavioral. Sometimes, it is good to have anxiety because it encourages us for survival, it leads us to concentrate on our concern to protect our self from danger or harmful situations.

Various models are developed to evaluate different types of anxiety such as Social phobias, Specific phobias, Panic disorder and generalized anxiety disorder. There are two other conditions where anxiety disorder also present i.e., Obsessive-compulsive disorder (OCD) and Post-traumatic stress disorder (PTSD). Every model has some advantages and disadvantages over other paradigms. Various parameters are involved which are used to evaluate if a drug or compound carrying an anxiolytic effect or not. Some paradigms such as the modified hole board test can be used to evaluate more than one type of anxiety disorder. Whereas, some paradigms such as marble burying behavior model is a point of argument that only the burying of marble cannot be calculated as a parameter of anxiety because it can be possible that in search of food, mice dig the bedding and as a result, marble buried due to digging.

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