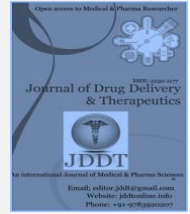


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Review Article

## Animals Use to Find Anxiolytic Activity: An Updated Review with Advantage of Each Model

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### Abstract

Anxiety affects both humans and animals in profound ways, and understanding its psychological and biological components is essential to the development of anxiolytic medications. Animal models are utilized as simulations to explain emotional activities and as screening tools in anxiety research. Rats are the animal model used most often; mice, a more genetically flexible mammal, do less well in animal models. Behavioral investigations need careful consideration of changeable characteristics related to handling, climate, or models. To help us better understand the neurobiological elements of anxiety, the most recent experimental anxiety models such as the elevated plus maze apparatus, light-dark model, open field apparatus, and hole board apparatus are being examined. Psychopharmacological disorders affect one in seven Indians; nevertheless, adverse effects include weight gain, cramping in the muscles, dysphoria, upset stomach, blurred vision, and issues with blood tests may occur. In order to improve our knowledge of the animal models used to determine the anxiolytic action in mice, we will talk about pertinent animal models that have been established in this article.

**Keywords:** Anxiolytic activity, Anxiety, Elevated zero maze, Plus maze, Elevated T maze, light and dark box model and Mirrored chamber, Animal models

## INTRODUCTION

Anxiety is a disorder that affects the mind, body, and emotions. It is also a psychological and physiological condition. It disturbs a person's regular life and is frequently linked to anxiety or worry. Acute fear states like anxiety are defined by motor sympathetic syndromes that cause alertness, anxiety, and hyperactivity. An acute stress reaction marked by abnormally high or abnormally low levels of anxiety or arousal is the most frequent observation. Inhibitory and facilitatory processes that either support or oppose anxiety states regulate anxiety states.<sup>1,2</sup> Fear is a defensive mechanism that enables people to pull away from dangerous circumstances. Long-term presence in improper settings interferes with everyday tasks and may result in the diagnosis of anxiety disorder. Separation anxiety, selective mutism, specific phobia, social phobia, panic disorder, agoraphobia, generalized anxiety disorder, obsessive-compulsive and associated disease, and trauma and stressor related disorder are among the main categories of anxiety disorders. Anxiety disorders can present with symptoms other than fear. The underlying link between the illnesses, as well as how similar their symptoms are and how frequently they occur, are used to classify them. Neurobehavioral animal models have proven invaluable in deciphering the etiology of illnesses and identifying cutting-edge pharmaceutical approaches for their management. But scientists contend that animal behavior differs from human behavior, and that actions deemed symptomatologically comparable may not necessarily be

tolerated. Three forms of validity are sought after in animal models of emotional disorders: conceptual validity, predictive validity, and facial validity. With several neurochemical systems and etiologies, anxiety is a complicated condition. Since it is difficult to create unique animal models for every anxiety illness, several animals can be utilized to evaluate the effectiveness and mechanism of action of compounds. This study centers on mouse models of anxiety-related diseases, which are categorized according to the type of unpleasant stimuli they encounter—for example, taught behaviors or intrinsic behaviors. These models, which include the Geller-Seifter and Vogel conflict tests, may be divided into conditioned and unconditioned reactions.<sup>3</sup> Anxiety disorder symptoms are mediated by neurochemical and neuropeptide processes in the cortex and subcortical brain. Since they have more than 90% of the same genes, animal models are useful tools in the biological sciences. Anxiety-related animal models attempt to capture elements of the aetiology, symptomatology, and management of anxiety disorders. They can serve as therapy models for human anxiety that is controlled by medication. These models are mostly used to find new anxiolytic drugs and investigate their processes. The biological environment in which animal models of anxiety are to be assessed is provided once their validity has been determined.<sup>4</sup> A recognized, distinct, non-conflicting threat is what causes anxiety, and a comparable warning signal is what causes dread. Clinicians distinguish between two types of anxiety: pathological anxiety is an improper reaction to internal or external stimuli, whereas

normal anxiety is an optimal response to stressful events. It is unlikely to construct comprehensive animal models that adequately reflect human characteristics due to the complexity of anxiety disorders and their comorbidity with major depressive disorder (MDD). Better definition and expansion of current models as well as novel experimental configurations that take into account a variety of elements in influencing anxious behavior are still possible, nevertheless. The article's examples simulate common anxiety symptoms rather than those of depressive illnesses.<sup>5</sup>

Additionally, when studying the effects of stress in humans is impractical or unethical, animal models can be very useful. However, choosing which biological correlates to study is not easy due to the limitations of animal models of human psychological disorders. These include:

- (i) Distinctions between the nervous systems of humans and non-human animals;
- (ii) Difficulties in determining common behaviors among species; and
- (iii) The demand that conclusions be applied to people based on animal examples.

These issues most likely point to a wide range in the origin and intensity of behaviors associated with anxiety.<sup>6</sup>

Preclinical studies on behavioral pharmacology have been made easier with the development of animal models of anxiety disorders. This phrase is deceptive, though, as it suggests anxiety is a single emotional state, and tests frequently miss new medications' anxiolytic effects. The capacity to identify the pharmacological activity of benzodiazepines (BZs) and similar chemicals is what led to the creation of experimental test techniques, which were sparked by the discovery of BZs fifty years ago. Clinical professionals later learned that BZs might cause addiction in patients, which prompted them to concentrate on non-benzodiazepine anxiolytics. The validity of the current screening methods was called into question by these new medications, underscoring the need for more precise and potent therapies for anxiety disorders. Conventional screening methods in animals, especially conflict tests in rats, failed to detect the anxiolytic potential of buspirone, a therapeutically effective partial agonist of the serotonin (5-HT) 1A receptor. As a result, unconditioned conflict tests such as the raised plus-maze were created. There are several types of anxiety, such as "pathological" or "trait" anxiety and "normal" or "state" worry. Anxiety is not a single, monolithic phenomena. Instead than being viewed as an excess of typical worry, pathological anxiety has a distinct neurological cause. Pharmacological treatments for human anxiety disorders have varying degrees of sensitivity to them. Animals used in experimental paradigms are frequently subjected to internal or external stimuli that cause anxiety. Anxiety without pathological anxiety is studied using animal models of state anxiety, as reported by Lister. These models only get more anxious in response to an external anxiogenic stimuli; their anxiety is normal at that particular instant. These models are crucial for neurobiological and neuropharmacological research, despite difficulties in animal investigations. By simulating elements of the etiology, physiology, and behavioral expression of fear and anxiety, they aid in the understanding of the neurological underpinnings of these emotions. A bewildering range of experimental techniques, with over 30 behavioral paradigms claiming face, concept, and predictive validity as animal models of anxiety disorders, is presented in the current literature.<sup>5</sup>

## Standards of validity for anxiety disorders in animals

In order to ease preclinical research on the behavioral pharmacology of anxiety, animal models of anxiety have been established. This phrase is deceptive, nevertheless, as it suggests that anxiety is a single emotional state and that new medications' anxiolytic effects are not always detectable by testing. The capacity to identify the pharmacological activity of benzodiazepines and similar substances was the primary driving force behind the creation of experimental test techniques, which were sparked by the discovery of BZs almost half a century ago. Later, doctors learned that BZs might cause addiction in patients, which increased interest in non-benzodiazepine anxiolytics.<sup>5</sup>

There are several types of anxiety, such as "pathological" or "trait" anxiety and "normal" or "state" worry. Anxiety is not a single, monolithic phenomena. Rather from being merely an excess of everyday anxiety, pathological anxiety has a distinct neurological cause. It has been demonstrated that various human anxiety disorders respond differently to pharmaceutical therapy. The majority of experimental paradigms include exposing animals to internal or exterior cues that are thought to cause anxiety. These animals are known as animal models of state anxiety because, at any given time, they exhibit typical anxiety; an external anxiogenic stimulation only amplifies their emotional state. Animal Models Have Proven Invaluable For Neurobiological And Neuropharmacological Research, Despite These Issues. Studies Using Animal Models That Mimic Several Features Of The Assumed Genesis, Physiology, And Behavioral Expression Of Fear And Anxiety Have Contributed Significantly To Our Understanding Of The Neurological Foundations Of Anxiety. Over Thirty Behavioral Paradigms Claim Face, Construct, And/Or Predictive Validity As Animal Models Of Anxiety Disorders; Yet, a Scan Of Recent Research Indicates a Bewildering Array Of Experimental Methodologies.<sup>5</sup>

## Clinical Categories of Anxiety

- ✓ Extreme anxiety that lasts all the time and has no obvious reason or focus is known as generalized anxiety disorder. One key feature of this type of anxiety is chronic terror.<sup>7</sup>
- ✓ Severe dread combined with distinct physical symptoms such sweating, tachycardia, chest aches, shaking, choking, and sudden, recurring panic attacks is known as panic disorder. There is usually a general component to this anxiety condition.<sup>8</sup>
- ✓ Post-traumatic Stress Disorder is a state of worry brought on by a persistent recollection of terrible events in the past.<sup>9</sup>
- ✓ Social anxiety the hallmark of disorder is a strong, acute anxiety of performance settings when one feels like they may become the center of attention and say or do something unpleasant or degrading. The scenario that causes this anxiety, like public speaking, could be quite specific to it. A phobia is an intense aversion to a particular item or circumstance, such as snakes, public places, flying, or social circumstances.<sup>6</sup>

## Experimental Animal Models for Anxiety

Animal models are the mainstay of preclinical research on the neurobiology of mental diseases. They are employed as simulations for underlying mechanism investigations as well as screening tools for the search for new treatment drugs.

There are two main subclasses of animal behavioral models of anxiety: 1) conditioned models 2)unconditioned models. The animal's responses to unpleasant and occasionally painful experiences—such as exposure to electrical foot shock—are taken into account in the conditioned model. The automatic or

natural reactions of the animal (such as flight, avoidance, and freeze) to stress stimuli that do not directly involve pain or discomfort are included in the unconditioned models (e.g. Introduction to a new, highly lighted test chamber or a predator).<sup>6,3</sup>

TABLE 1: TYPE OF ANXIETY MODELS<sup>6,3</sup>

Conditioned Models	Unconditioned Models
Four plate method	Open field model
	Hole Board Apparatus
Fear-potentiated startle	Elevated plus maze
Vogel water-lick conflict Test	Elevated zero
	Maze
	Elevated T maze
	Social Interaction Test
	Marble Burying test
	Novel object Recognition test
	The light-dark box
	Hole dipping curiosity test
	Mirrored chamber

## CONDITIONED MODELS

### FOUR PLATE METHOD

In a behavioral research called the four-plate test (FPT), mice are not allowed to explore new habitats. There are four identical metal plates that make up the floor, and depending on where the quadrant crosses, there are small electric foot shocks. Every time the mouse moves from one plate to another, the entire floor is electrified to restrict its exploratory tendency and trigger a basic flight response. Before making judgments about a drug used in this test, it is imperative to confirm that it has no analgesic effects. Benzodiazepines (BDZs) increase the frequency of penalized animal-accepted crossings. Analyzing the underlying processes of anxiety, such as the inter-regulation of noradrenergic alpha2 receptors and the 5-HT2 subtype, is made easier by the FPT. At intervals of 24 hours to 42 days, punishment retest responses are reduced by one prior undrugged FPT exposure. Furthermore, like with the Elevated plus maze and Light and Dark Model, past experience reduces the anxiolytic response to the BDZs lorazepam and diazepam. The validity of the FPT is shown by its performance in our laboratory and the evidence of an anxiolytic-like effect of ADs in this model, despite its limitations. It makes it possible to investigate the underlying processes of anxiety, such as the interplay between a noradrenergic and 5-HT2-subtype receptors. Nowadays, the FPT is being utilized more and more to identify possibly novel anxiolytic medications' anti-anxiety properties.<sup>6,10</sup>

### FEAR-POTENTIATED STARTLE

The two stages of the Pavlovian fear conditioning method, which was created by Brown et al. in 1951, include teaching animals to link a neutral stimuli with an unpleasant stimulation—such as an electric foot shock—and then exposing them to a loud noise. The simultaneous presentation of the previously conditioned light stimulus amplifies the startle reaction to this unconditioned stimulus. It is possible to see this potentiation even a month after training. Anxiolytics cause the startle amplitude to decrease dose-dependently without raising the baseline startle level. A non-specific locomotor

impairment would be revealed by a decline in the baseline. The primary findings of this paradigm were reported by Davis et al., and they show that buspirone-like medications and benzodiazepines reduce fear-potentiated startles, frequently with no alteration to the baseline response. Nevertheless, in this model, the administration of some antidepressants, including imipramine, fluvoxamine, or amitriptyline, appears to have no impact.<sup>6,10,11,12</sup>

### VOGEL WATER-LICK CONFLICT TEST

The test, which was created by Vogel et al., is a well-known method for identifying anxiolytic action similar to diazepam in rats. A waterspout rewards thirty animals with water, but in exchange, they get a little electric shock to the mouth. The anti-conflict impact of diazepam and pentobarbital was strong, as evidenced by the increased number of electric shocks administered to the mice throughout the test session. Geller and Seifter created the exam first, while Vogel later made modifications. It has a strong predictive ability for anxiolytic medications. Rats used in this experiment are trained to press a lever to get a liquid sweetened with sugar at different intervals after being denied food for a whole day. An electric shock is used as a punishment for the lever push action, as demonstrated by a signaling stimulus. This creates a conflict between the rewarding behavior of drinking the tasty water and getting shocked. Under normal circumstances, the impulse to pull the lever lessens; however, the effects of anxiolytic medications are the reverse, making punishment more likely. Analyzing medications also have anti-conflict qualities in this scenario, which results in more licks that are penalized.<sup>3,6,13</sup>

## UNCONDITIONED MODELS

### Open Field

The Hall test was created for rats and entails putting the animals in an unfamiliar environment to watch for a variety of behaviors, including thigmotaxis (nervous behavior), urine, and feces. Each animal is examined separately on a square-shaped open field floor. Anxiety behavior in the open field is caused by agoraphobia and individual testing. The ratio of squares visited in the center to those on the periphery should decrease with higher anxiety levels. With respect to criteria including time spent in the center area, movement, time spent motionless, fecal boil, rearing and grooming, the test assesses both exploratory behavior and aversion towards the central region. The open field floor is currently mainly partitioned into squares. Each animal utilized for experimentation is always placed in the same manner. A simple open-field arena serves as the experiment site. The bottom surface is divided into equal-sized square grids, the inside walls and bottom squares are darkened, the squares along the walls represent the periphery region, and the remaining squares represent the core area. Agoraphobia and individual testing are the two factors that might induce uneasiness in the open environment. With increased worry, the "number of squares visited in center/number of squares visited on periphery" ratio should mostly decline. The test is based on the exploratory behavior and the aversion to the centrally lit zone. The features that are measured include time spent in the center, distance traveled during locomotion, time spent still, number of fecal boils, number of raisings, and number of groomings. Neophilia, a disorder where a person spends more time in the center, is brought on by anti-anxiety drugs.<sup>3,14,15</sup>





FIGURE 1: OPEN FIELD TEST

#### HOLE BOARD TEST

The hole board exploration paradigm was developed by Boissier and Simon to examine the anxiolytic properties of medications in mice. The head-dipping habit of certain animals, which is seen as a sign of neophilia, served as the basis for the model. The amount of head dips is negatively correlated with the anxiety level. Since the hole board model measures fearfulness rather than novelty, researchers have begun to use it to evaluate medications for anxiety. The purpose of the hole board test is to evaluate certain head dipping behaviors. The hole board equipment was an open field with sixteen uniformly spaced holes in the floor measuring three centimeters in diameter. There were four groups of six Swiss Albino mice apiece. The extracts were administered 45 minutes prior to testing, while the conventional drug, diazepam, was given 30 minutes beforehand. The mice explored the board and dipped their heads into the holes after being placed in separate corners. Every five minutes, the number of head dips and sections crossings made by each mouse was tallied.<sup>3,10</sup>

#### ELEVATED PLUS MAZE

An extensively used animal model for researching anxiety in rats is the elevated plus maze (EPM). Based on mice' innate curiosity about new places and aversion to exposed, bright, or lofty situations, it has two closed arms and two open arms positioned perpendicularly. The EPM test involves placing mice in the middle of the labyrinth, facing open arms, then elevating them by 50 cm for an hour in order to evaluate the anxiolytic impact. Measures are made to ascertain the total number of entries in open arms and the average amount of time spent in open arms during the test period. The total number represents the rodent's overall movement, whereas the quantity of entrances and the amount of time spent in open arms show non-anxious behavior. Video recording, with or without software, is typically conducted because the animals' behavior can be affected by the experimenter's presence. Within five minutes of the animal being placed within the shuttle box arch, the experimenter notes the animal's behavioral changes. The amount of time spent in light, the percentage of horizontal and vertical motions in light, and the quantity of shuttles are the assessment indicators. Anti-anxiety medications have been shown to lengthen the animal's stay in the light and increase the number of times it shuttles through the box. Baseline values can also be impacted by other elements including lighting, how the animals were handled before the test, and the state of the habitat. Benzodiazepines and other anxiolytic medications raise the proportion of people entering and staying in open arms, which raises the model's sensitivity even further. Confinement with open arms results in physiological signs of stress, although exposure to conventional anxiolytic drugs enhances the exploration of arms. The animals in the EPM exhibit basal behavior that is influenced by a variety of factors, including housing circumstances, illumination levels, circadian cycle variability, prior handling or stress exposure, and familiarity with the labyrinth. Individual accommodations make rats feel more anxious, whereas mice feel less anxious; this might be because different groups have different social organization patterns. Re-exposure to the EPM causes a significant reduction in the open arm's exploratory activity and may even eliminate the benzodiazepine's anxiolytic effects.<sup>3,10,15,16</sup>

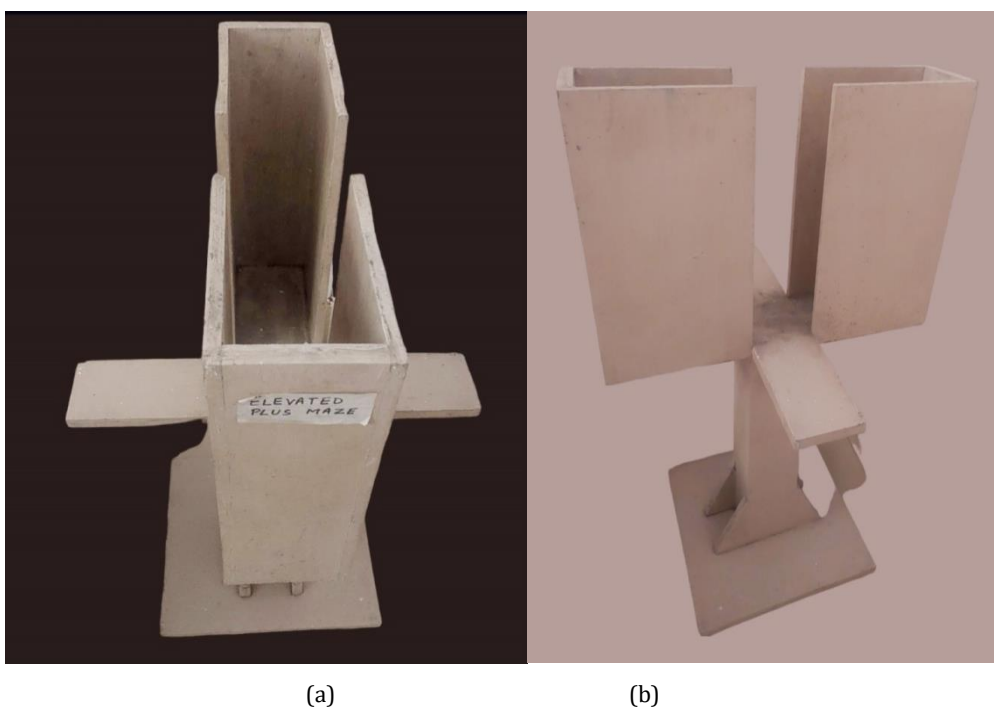


FIGURE 2: ELEVATED PLUS MAZE

### ELEVATED ZERO MAZE

The Elevated Zero Maze (EZM) is a modified version of the Elevated Plus Maze (EPZ) that incorporates traditional and novel ethological measures for drug effects analysis. The EZM is a circular runway with open and enclosed areas, allowing rodents to explore the maze continuously. The uninterrupted nature of the open versus enclosed segments alleviates issues with the center zone of the EPM. The anxiety index is related to the percentage of time spent and entries in the open areas during a 5-minute session. Anxiolytics like diazepam and chlordiazepoxide significantly increase the percentage of time spent in the open quadrants and other ethological measures, such as head dips and reduced stretched attend postures in the enclosed. Videotaping of the session is recommended to minimize environmental variables that may impact anxiety-like behaviors. The EZM is designed to eliminate the central square in traditional EPM and allows rodents to continuously explore the maze.<sup>3,6,17</sup>



FIGURE 3: ELEVATED ZERO MAZE

### ELEVATED T MAZE

Based on the EPM, the elevated T maze (ETM) is a model with three arms: two equal-sized open arms on either side and one arm surrounded by a lateral wall. The conditioned reaction, which is represented by the animal's inhibitory avoidance of the open arms, and the unconditioned response, which is represented by the animal's escape behavior when it is put at the extremity of these arms, may both be measured in the same animal using the ETM. Panic disorders and generalized anxiety have been linked to these reactions. The development of the ETM was prompted by discrepancies observed in existing animal models of anxiety, specifically the EPM, with reference to medications that directly disrupt serotonergic neurotransmission. Rats have two defensive mechanisms: one-way escape, which symbolizes unconditioned fear, and inhibitory avoidance, which reflects conditioned fear. One of the closed arms of the EPM is closed to create the T maze. The mouse is subjected to one of the open arms for thirty minutes before to the trial as part of a preexposure exercise, which reduces withdrawal latency and increases drug sensitivity. The avoidance task shares a pharmacological profile with generalized anxiety disorder (GAD), but the one-way escape task is thought to represent a model of panic disorder.<sup>3,6,15</sup>



FIGURE 4: ELEVATED T MAZE

### HOLE DIPPING CURIOSITY TEST

Mice are curious in the hole dipping test, which lasts for five minutes. During that time, the animals peer through the hole, cut a photocell, and the number of cuts is recorded. Evaluations include placing albino mice individually on a wooden board with 16 uniformly spaced holes, 30 minutes after being injected with saline, diazepam, or a test medication. The number of times the mice dip their heads into the holes over the course of a five-minute trial is counted. Depending on the type of test medication, it is typically seen that there is either a considerable increase or decrease in the head dip reactions in mice.<sup>6,18</sup>

### SOCIAL INTERACTION TEST

The social avoidance and social terror behaviors that the rats exhibit when they are introduced to unknown pairings of male or female mice in a novel setting are the basis of File and Hyde's test. These are examples of the two categories of symptoms of social anxiety disorder: particular and broad. Social fear manifests in behaviors like flight, protective burying, and alarm screams, whereas social avoidance is characterized by a decrease in social interaction time. Aversive stimuli, which are frequently included in other anxiety models, are not necessary for this test. The anxiolytic impact is deduced from the increase in interaction time between the pairings. Four sets of six Swiss albino mice each are created from mice weighing between 20 and 30 grams. The extracts were administered 45 minutes prior to testing, while the conventional medication, diazepam, was given 30 minutes beforehand. Prior to the study, they were kept in isolation for five days with unrestricted access to food and drink. They were routinely handled and weighed throughout this period. To guarantee that every rat had an equal amount of exposure to the different light levels, the positions of the cages inside the rack were changed. The mice were randomized at random to the "low light and unfamiliar" test conditions. The text box was 22 by 15 by 12 centimeters. It was filled with pairs of mice, and for five minutes, their behavior was observed. It was noted that they would sniff, nibble, groom, follow, mount, kick, box, wrestle, and leap on, under, or over their buddy.<sup>3,10</sup>

### MARBLE BURYING TEST

The behavioral model used in studies on obsessive compulsive disorder is the Marble Burying Test. Neophobia is the dread of

unfamiliar or odd items; when rats are exposed, they exhibit certain behaviors such as digging, burrowing, and rearing. The bedding material in the cage should be inserted 5 cm deep, and the marbles should be spaced equally apart. The animal is put in its cage and given a half-hour. The study reveals that the number of marbles buried decreases in animals receiving medication, and the type of bedding used also affects the results. Some studies have reported an increase in marble burial in areas with poor bedding density. The behavior of marble burying (MB) was evaluated using established techniques. The testing setup comprised a 33 cm long by 21 cm wide by 19 cm high plexiglass mouse cage that was filled with corncob bedding (Rahi Agro Industries, Ahmedabad, India) to a depth of 5 cm. The cage was then placed in a sound-absorbing room that was lit with white light. Ten transparent glass marbles with a diameter of 10 mm were uniformly distributed and laid across the bedding surface in the shape of a grid before each test. Every mouse was then put into the observation cage. The mice were carefully taken out of the chamber after the 60-minute test, and the number of marbles that had been buried (meaning that at least 50% of them were hidden by bedding) was counted.<sup>3,10</sup>

#### NOVEL OBJECT RECOGNITION TEST

Ennaceur and Delacour initially suggested this test, which is based on the differential investigation of known and unknown objects. The benefit is that, unlike the visual recognition test in subhuman primates, it is based only on working memory and does not require any unpleasant stimuli, such as electric shock. The animal is given thirty to sixty minutes to investigate its surroundings before the experiment day. In the first trial, the mouse is exposed to the novel object when two comparable novel objects are positioned in the center of the device. In the second trial, two objects—one familiar and one unfamiliar—are put, and the frequency and length of exploration behavior—such as touching, licking, and biting the unfamiliar object—as well as avoidance behavior—such as spending time in the apparatus's periphery—are noted. The trials may go for ten to thirty minutes. This gauges rats' approach-avoidance behavior. Anxiolytic medications reduce avoidance behavior and lengthen the time spent exploring a novel thing.<sup>3</sup>

#### PREDATOR STRESS MODEL

Rats are prohibited from physical contact or assault, but they are subjected to auditory, visual, and smell cues linked to the predators as part of the study design. In response to predator aggressiveness or smells, rats exhibit anxiety-like responses such as running away, crouching, stiff movements, and noticeably increased excretion. Corticotrophin-releasing hormone (CRH) mRNA expression is upregulated in response to both acute and chronic predator stress, indicating that predator stress triggers anxiety-inducing behaviors via triggering the hypothalamus-pituitary-adrenal axis.<sup>16</sup>

#### THE LIGHT-DARK BOX

The light-dark exploration test, developed by Crawley & Goodwin in the early 1980s, is a novel experiment that measures rodents' natural tendency to explore a novel environment against the aversive properties of brightly lit open fields. The test involves two separate compartments: protected (dark) and unprotected (lit). The test creates an intrinsic conflict between the rodents' exploratory drive and their avoidance of the illuminated compartment. Treatment with anxiolytic medications like benzodiazepines increases the time spent in the illuminated compartment and the number of changes between the two regions. The two compartment exploratory models have been validated pharmacologically, behaviorally, and physiologically. The time spent in the light area is the most reliable parameter for assessing anxiolytic activity. After administering the test drug, each animal was placed in the center of the light compartment for 30 minutes, and the number of transitions and time spent in the light zone were noted.<sup>6,10,19,20</sup>

#### MIRROR CHAMBER TEST

Placing a mirror in an animal's surroundings causes many of them to react with approach avoidance. Rats exhibit a prolonged delay to enter a mirrored chamber device that Toubas (1990) devised. New stimulation provokes conflict avoidance and exploration at the same time, leading to approach avoidance behavior. The anxiety analogy uses the prolonged latency to enter the chamber of mirrors as a metric. Anxiolytic benzodiazepines, including triazolam and diazepam, considerably lower this latency in a dose-dependent way. A mirrored cube that is open on one side is the main component of the mirror chamber equipment, which is a square plexiglass box. The 30 x 30 x 30 cm mirrored cube is made up of five pieces of mirrored glass, one side painted dark brown and the other mirrored. The inside surface of the cubical mirrored chamber was composed of mirrored glass. The 40 x 40 x 35.5 cm container box features opaque black walls and a white floor. When the mirrored cube is placed in the middle of the container, a 5 cm corridor surrounds the mirrored chamber entirely. On the wall of the container, a sixth mirror is positioned to face the one exposed side of the mirrored chamber. The reason for this decision should be the investigator's background and understanding of the model's shortcomings. Special consideration should be given to procedures that may track bias resulting from regional laboratory circumstances as well as false-positive or false-negative results. Some of these topics were covered in the most recent analysis. Animal models are a useful resource for researching the neurobiology of illnesses related to stress and anxiety, despite several limitations. All of the container walls are black, with the exception of one single mirrored section. To prevent habituation issues, rats are only tested once after being exposed to the chamber of mirrors. This approach focuses on calculating the latency from the surrounding corridor to the chamber of mirrors.<sup>21,22</sup>

**Advantages<sup>3,6,23</sup>**

Anxiety model approaches		Advantages
Unconditioned models	Open field model	1. Easy to understand experimental procedure 2. Less damage to animals. 3. Intuitive reaction to conditioned animal behavior 4. Results are more realistic and objective when they are semi-automated. 5. Practical and effective
	Hole Board Apparatus	1. utilized just to investigate exploration and/or evaluate potential medication effects on mice' exploratory behavior in this setting
	Elevated plus maze	1. Easy to understand experimental procedure 2. Less damage to animals. 3. Intuitive reaction to conditioned animal behavior 4. Results are more realistic and objective when they are semi-automated. 5.The advantage of testing anxiety and learning/memory at the same time and in the same animals
	Elevated zero Maze	1. Easy to understand experimental procedure 2. Less damage to animals. 3. Intuitive reaction to conditioned animal behavior 4. Results are more realistic and objective when they are semi-automated.
	The light-dark box	1.easy to complete 2. simple to understand 3. animal training is not required
	Novel object Recognition test	no need of any aversive stimuli like electric shock
	Predator stress model	1. Comparable to the symptoms of anxiety disorder clinically 2. Less damage to animals 3. Less time-consuming
	Vogel water-lick conflict test	Avoiding a prolonged training period

**CONCLUSION**

Since anxiety models are sensitive to environmental changes and need to be carefully chosen in order to investigate novel chemicals or comprehend anxiety pathways, there is no correlation between clinical profiles of anxiety disorders and anxiety models. The development of models based on individual sensitivity and genetic or epigenetic variables will be critical to the future of novel medications for anxiety disorders. Compared to fifty years ago, there are a lot more animal models of stress and anxiety, which makes it challenging to choose the best model for research. Special consideration should be given to procedures that may track bias resulting from regional laboratory circumstances as well as false positive or false negative results. Animal models are useful tools for researching the neuroscience of anxiety and stress-related illnesses, despite several drawbacks.

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