International Journal of Basic & Clinical Pharmacology
Desingu R et al. Int J Basic Clin Pharmacol. 2023 Jan;12(1):134-141
http://www.ijbcp.com
pISSN 2319-2003 | eISSN 2279-0780

DOI: https://dx.doi.org/10.18203/2319-2003.ijbcp20223368

Review Article

Animal models of anxiety: a review

Ramyaa Desingu*, Balakrishnan Sadasivam, Shamsher Singh Kalra, Bhawna Lakhawat

Department of Pharmacology, All India Institute of Medical Sciences, Bhopal, Madhya Pradesh, India

Received: 13 November 2022
Accepted: 03 December 2022

***Correspondence:**
Dr. Ramyaa Desingu,
Email: docramyaa@gmail.com

ABSTRACT

Anxiety is a disorder that affects the quality of life and also imposes a huge economic burden. Animal models of anxiety have widened our understanding of the pathophysiology behind anxiety and to identify newer pharmacological compounds. Every behavioral test has its own limitations, and there are ways to minimize these like environment and handling. This review lists various experimental models of anxiety based on unlearned ethological models, learned responses like the Vogel conflict test, and psychological and physical stress models.

Keywords: Anxiety disorders, Anxiety models, Stress, Animal models

INTRODUCTION

Fear is a normal behavior which is protective in a way that allows individual to withdraw from a threatening situation.[1] When this is present in inappropriate situation and for a longer duration, it affects the daily activities of the life, and the diagnosis of anxiety is made. According to DSM 5 anxiety disorder is classified into 3 major types. Anxiety disorders which consist of separation anxiety, selective mutism, specific phobia, social phobia, panic disorder, agoraphobia, and generalized anxiety disorder, obsessive-compulsive and related disorder which consist of body dysmorphic disorder, hoarding disorder, excoriation disorder, the third type is trauma and stressor related disorder which consist of post-traumatic stress disorder, acute stress disorder and adjustment disorder.[1] Fear is not the only symptom in anxiety disorder as seen by the above classification. This classification is based on the underlying relationship between the disorders and their similarity in symptoms and the co-occurrence among them.

Neurobehavioral animals model have been vital in understanding the pathogenesis of a disorder and discovering novel pharmacological strategies to treat them. But still these models are under argument that animals do not behave in same as humans and various behaviors which are considered similar to symptomatology of a disorder is not always accepted by the researchers at all times. Still animal models of emotional disorders try to obtain three types of validity; face validity is the ability to reproduce features of psychiatric disorder, the ability to detect clinically effective pharmacological agents shows the predictive validity, and the similarity in the etiology of the disease in humans and animals gives the construct validity.[2]

Anxiety is a complex disorder with different etiology and involves different neurochemical systems. Animal model for specific for each anxiety disorder are difficult to develop, hence various models can be used to screen a compound efficacy, and to assess the various mechanism by which it act.

This review will be focusing on rodent models of anxiety related disorders. The models are classified based on the nature of the aversive stimuli, whether they are innate behaviors like avoidance and freezing or a learned response following exposure to electric foot shock, based on this the anxiety rodent models into conditioned (e.g., Geller-Seifter and Vogel conflict test etc.) and

unconditioned responses (eg. Elevated plus maze, open filed etc.)

UNCONDITIONED RESPONSE MODELS

Elevated plus maze

EPM is one of the most commonly used model in evaluating the anxiolytic effect of a drug. In a study by Montgomery et al who investigated whether novel exposure would stimulate fear as well as exploratory drive.[3] He identified that approach avoidance conflict behavior was observed when exposed to novel stimulation, increased fear was seen with elevated alleys than closed alleys, strength of fear decreased with time and after a period of non-exposure the strength of fear recovers.[4] It was further validated by Handley and Mithani for use in rats and mice.[4] EPM apparatus consist of two open arms and two closed arms raised above a level from ground and are perpendicular to each other.[2] The EPM arms are usually 30 cm long 5 cm wide, elevated about 40-60 cm from the ground level.[5] This test is based on the rodents tendency to avoid elevated places and explore novel environment.[2]

The number of entries and time spent in open arms are indicative of non-anxious behavior and total number of entries indicate the general locomotion of the rodent.[6] The rodent is usually placed in the centre square facing the open or closed arm, and the activity is recorded for 5-10 min.[5] The presence of experimenter in the study can affect the animals behavior, hence video recording with or without the use of software is usually practiced.[2,5] Other factors like lighting, handling of animals prior to the test, and housing condition can affect the baseline values.[2] Anxiolytic drugs like benzodiazepines increase the percentage entry and time spent in open arms, further to increase the sensitivity of the model ethological behaviors like head dipping in open arm for detecting non-benzodiazepines anxiolytics can also be studied.[2]

Elevated T maze and zero maze

Graeff et al initially proposed the elevated T maze.[2] Handley who proposed EPM found inconsistencies with the drugs that alter 5-HT transmission, because it is a mixed model.[7] Rodents display two defense strategies, when the animal is present in the closed arm it avoids open arm and escapes to closed arm when present in open arm.[7] This behavior task that is inhibitory avoidance which represent conditioned fear and one way escape represents unconditioned fear and this can be separated by elevated T maze.[7,8] The T maze is produced by closing one of the closed arm of EPM.[8] Before the day of experiment pre-exposure task is done by exposing the rodent to one of the open arm for 30 min, this decreases the withdrawal latency and increases the drug sensitivity of the escape arm.[2] After pre-exposure the inhibitory avoidance task is recorded by placing the rodent in closed arm and the latency to withdraw from this arm is recorded, the number of trial varies and trial interval is usually of 30 sec.[7] The rodent is placed in open arm to record escape latency.[2] The pharmacological profile of avoidance task is similar to generalized anxiety disorder (GAD) and one way escape is considered as a model of panic disorder (PD).[7]

Elevated zero maze is a circular platform with two enclosed quadrants and two open quadrants, it is designed in a way to eliminated the central square which is present in traditional EPM and allows the rodent to continuously explore the maze.[9,10] The time spent in open area and number of entries in open arm is recorded for 5 min.[2] Ethological behavior like head dipping over the edge of the platform and stretched attend posture can also be included, anxiolytics like diazepam increased the ethological measures and time spent in open quadrants.[11]

Light– dark box

Crawley and Goodwin developed the light dark box before the EPM.[2] It is based on the ethological behavior of exploring and retreating from unknown spaces.[12] The advantages of this model is it is simple to perform, no need of prior training of animals and easy to interpret.[12] The apparatus consist of light and dark chambers, which is 44×21×21 cm in dimension one third is dark and two third is open in the top and illuminated by the room light, the two chambers are connected by a shutter which is 13 cm long and 5 cm high.[12] The preference for dark chamber increases when the animal is anxious.[5] The animal is placed in dark chamber initially to acclimatize. The parameter observed in this model is latency to initial transition into light chamber, this is observed after removing the shutter in the connecting door.[2,5] The time spent in light chamber can also be recorded, drugs having anxiolytic effect increases the time spent in light chamber.[2]

Open field test

Open field test was initially designed by Hall S, where he measured defecation and urination to correlate with differences in emotionality.[13] The apparatus initially used was a raised platform which did not have surrounding walls, later the open field apparatus had surrounging walls of about 10 cm height and the arena with wide range of dimensions, 10×20 cm, 20×40 cm, 40×40 cm, and even 100×100 cm and this is equally divided.[14] The animal is placed on the centre of the arena and test is conducted for 5-10 min.[5]

The rodent initially shows a behavior called thigmotaxis, that is aversion to central area of arena and they tend to move towards the wall.[5] The test is based on the exploratory behavior and aversion towards central illuminated area.[15] The parameters measured are, time spent in central area, locomotion measured by distance travelled, time spent immobile, number of fecal boil, number of rearing and grooming. The antianxiety drugs increase the time spent in central area, which is considered as neophilia.

Hole board test

Boissier and Simon introduced hole board exploratory model initially for mice to test the anxiolytic character of the drug.[14] The apparatus is a square board with 16 holes each of them around 3 cm in diameter, or other modifications in this dimensions are also used by researchers, which consist of holes, 4 cm diameter, and arena measuring 68×68 cm. The model is based on the specific animal behavior head dipping which is considered as a measure of neophilia.[5] The anxiety level is inversely proportional to the number of head dipping.[2] In order to overcome the deficiencies with the open field model, researchers started to use hole board model to screen the drugs for anxiety, commenting that open field is a measure of fearfulness rather than novelty, rather than novelty, because forcing animal in a closed environment does not always stimulated the exploratory behavior of rather, also increases the anxiety, which is correlated with the increased levels of corticosterone in rodents after exposure.[15] The head dipping behavior which is assessed in this model is considered a valid measure of neophilia.[15]

Social interaction test

This test was proposed by File and Hyde and it is based on two behaviors social avoidance and social fear which is shown by the rodents when they are exposed to unfamiliar pairs of male or female rodents in a novel environment.[16] This represents the symptoms of social anxiety disorder, which is of two types specific and general.[16] Social avoidance is seen as decrease in time in interacting and social fear is shown by behavior like flight, defensive burying, and alarm cries.[16] This test does not need aversive stimuli which is commonly a part of other anxiety models.[2] The increase in interaction time between the pairs is inferred as anxiolytic effect.[2]

Novel object recognition test

This test was first proposed by Ennaceur and Delacour, based on the differential exploration of familiar and novel object.[17] The advantage is it does not need any aversive stimuli like electric shock, similar to visual recognition test in subhuman primates and is based on pure working memory.[17] Before the day of experiment the animal is allowed to explore the environment for 30-60 min. Two novel objects which are similar are placed in the centre of the apparatus and the rodent is exposed to novel object in the first trial. In the second trial one familiar and one novel object is placed and the frequency and duration of exploratory behavior like touching, licking and biting the novel object are recorded, also avoidance behavior like time spent in the peripheral area of the apparatus are observed too.[17] The trials can be conducted for 10-30 min.[5] This measures the approach-avoidance behavior of rodents.[5] Drugs with anxiolytic effect increases the exploratory time of the novel object and also decrease the avoidance behavior.[18]

Marble burying test

Marbel burying test is the behavioral model for research of obsessive compulsive disorder.[19] Neophobia is fear of new or strange objects, upon exposure rodents shows specific behaviuor like burrowing, burying, digging and rearing.[20] The cage should be filled with bedding material 5 cm deep, and the marbles are placed in equidistance.[5] The animal is placed in cage and left for 30 min.[20] The marbles covered 2/3rd of its depth is considered buried, the number of marbles buried is counted at the end of the session.[20] Animals treated with drugs shows decrease in number of marbles buried. Other factors like the bedding material used, and the number of marbles used can affect the outcome of the study. It is seen in few studies that low density bedding showed increase in marble burying.

CONTIONED RESPONSE MODELS

Fear conditioning tests

Social fear and avoidance of social situations represent the main behavioral symptoms of social anxiety disorder (SAD) a disorder that is poorly elucidated and has rather unsatisfactory therapeutic choices. Therefore, animal models are needed to study the underlying cause of the disorder and possible novel treatment possibilities. Fear conditioning models involve the encoding of traumatic memories, representing a psychological stress without physical stimuli.[21] They have been associated with a vulnerability to phobic fears and other anxiety-related disorders, such as panic disorder (PD), agoraphobia, and posttraumatic stress disorder. In this model, administration of anxiolytic drugs immediately before the pairing of conditioned stimulus (CS) and unconditioned (US) (during the memory acquisition process) affects the formation of conditioned learning. If administration occurs before the re-exposure to CS, it will affect fear and anxiety expression acquired during the conditioning. The drug could also affect extinction of the conditioned response, where a new learning process (that the CS no longer predicts the occurrence of the UCS) occurs after repeated exposure to a CS in the absence of the US.[22]

The Geller-Seifter and Vogel conflict tests

This test was first developed by Geller and Seifter and later modified by Vogel. It shows a high predictive value for anxiolytic drugs. In this test, rats are deprived of food for 24 hours are trained to press a lever and obtain a sugar-sweetened drink at variable intervals (the non-punished component). In the test session, a signaling stimulus (such as tone or light) is introduced, showing now that the lever-press behavior will always yield a reward but, at the same time, will be punished by an electric shock, producing a conflict between drinking the palatable water and receiving the shocks. In normal conditions, the urge to press the lever decreases, but anxiolytic drugs show

opposite effects, and thus increasing the probability of punished responses.[23]

John Vogel in 1971, had done a more simplified test in which animals were deprived of water for 24 hours and then trained to find a water bottle in an experimental box. On subsequent day (after 24 hours of water deprivation), animals are re-exposed to a stainless steel grid floor. After 20 licks, animal receives a mild shock (0.5 mA). So, in this model, anxiolytic drugs also show anti-conflict properties, hence increasing the number of punished licks.[24]

ANIMAL MODELS OF STRESS

Psychological and physical stress models

Ideally these models should induce stress in rodents by exposure to physical or physiological stress. These procedures might be used in chronic or acute stress, depending on objectives and parameters chose to evaluate the effect of stress on anxiety. The main stress is psychological and physical stress. Psychological stress includes neonatal isolation, circadian rhythm change, predator tests. Physical stress includes restraint stress, immobilization stress, electric foot shock stress.[25]

PSYCHOSOCIAL STRESS

Predator encounter based models/ rat exposure tests

Defensive behavior is observed in all mammalian species and occurs in response to threatening conditions, like the presence of live predators and environmental hazards. Therefore, exposure to an ethological stimulus evokes defensive responses that resemble emotional states related to fear and anxiety. Accordingly, predator exposure constitutes an important animal model for identification of impact of threatening situations on different brain regions and relationship between defensive behavior and fear-related disorders, such as panic attacks and PTSD.[26]

This model was pharmacologically validated with the observation that chronic administration of panicolytic drugs decreases the fight reactions induced by the presence of the predator, whereas benzodiazepines preferentially inhibit the avoidance behavior. These latter effects were also described in cat odor models, as pretreatment with chlordiazepoxide reduced the subsequent anxiogenic-like behavior observed in the EPM and light-dark box. However, acute treatment with benzodiazepines did not reduce the defensive behaviors elicited by odor itself. On the other hand, other studies showed that this treatment is able to reduce risk assessment behaviors and increase approach to the odor.[27]

Neonatal isolation stress

In early stressful experiences, such as neonatal separation or maternal separation, have a deep impact on neural and behavioral aspects on further life quality. During this separation process, on second day after birth, the litter of inbred strain is removed and placed in other cage for one hour (9/12 am) in a room far away from animal facility. The white noise is being played in the background to mask up the sound of other pups. After one hour, litter is placed back in their original cages. The separation procedure is followed for eight days.[28] This model is extensively used to illustrate the effect of early lifetime stress on susceptibility to addiction and anxiety-like behavior which are usually seen in adult rodents subjected to fear conditioning, or social interaction tests.[29]

Stress induced by circadian rhythm changes

The circadian rhythm of an organism is an integral component of its homeostatic functioning. It is regulated via the hormone melatonin secreted from the pineal gland. Any alterations we bring about in this rhythm (either by altering the sleep- wake cycle or by reversing the lit/dim conditions in its environment) is definitely going to evoke a stressor response in it.[30] This can be consequently measured by the behavior of the organism in established tests or by quantification of biochemical parameters of stress. This test however has an associated caveat, it's good for acute stressor responses in rodents but long term subjection to altered circadian cycles shall lead to the animal adapting to the changed life conditions and the corresponding stressor responses shall fade away. Also, genetic disruption of circadian rhythms in the suprachiasmatic nucleus (SCN) causes helplessness, behavioral despair, and anxiety-like behavior in mice which is yet another way of studying this stressor phenomenon.[31]

Stress induced by a noisy stimulus

This model has a great parallelism between what we see in modern day human lifestyle and what we can experimentally demonstrate in rodents. Noisy Stimuli can be of 2 types: acute or chronic. Simply put, a sudden loud bang falls in the first category and a long-standing high amplitude sound stream falls into the latter. Rodent models can be similarly subjected to a sudden high amplitude sound from a loudspeaker placed at a certain distance in a particular direction from its cage to produce a acute noise-induced stressor response and they can also be subjected to continuous high amplitude sounds from a definite sound source for a pre-set duration per day and a particular number of days.[32] This shall mimic the chronic exposure of humans to noisy environment. Furthermore, the aforementioned animal models can be tested for anxiety/depression induced by the acute/chronic noise stressors in standard tests or quantification of their biochemical parameters of stress can be performed.[33]

Low temperature induced stress

This is a unique model for understanding the human behavioral and biochemical adaptation as well as

acclimatization to low temperatures. As for adaptation, chronic subjection to low temperatures which can be seen in residence at cold geographical zones can be studied in rodent models by placing them in an environment of low temperature (eg., 4°C for 15-30 min per day for 7-14 days). Also, for acclimatization studies, acute exposure of the rodent to low temperature (e.g. dipping the animal in water cooled to 15-18°C for 15-30 min) shall evoke a stressor response.[34] These rodents subjected to acute/chronic low temperature stressors can be analyzed for anxiety/depression in standard tests or the biochemical alterations in hypothalamus- pituitary- adrenal axis due to cold stimuli can be quantified.[35]

Stress induced hyperthermia

Stress exposure is frequently associated with an elevated body temperature which can be referred to by different names like emotional fever, stress-induced hyperthermia (SIH), etc. This can be corroborated by a rectal measurement of core body temperature of a mouse that induces a rise of 1-1.5°C over a time interval of 10-15 min. This phenomenon has been used to design a specific test for measuring SIH: the singly-housed SIH paradigm in mice. Here, measurements of mouse body temperature are done via the rectal route which, being anxiogenic, causes hypothalamo-pituitary-adrenal activation and concordant rise in the rodent's body temperature.[36]

More detailed analysis of the neurophysiology of stress generation, the cortical areas involved and biochemical quantification of markers of stress can be done by serial measurements of these values after a set starting point of evaluation.[37]

PHYSICAL STRESS

Restraint and immobilisation stress

This is arguably one of the most, if not the most, anxiogenic stimulus a human being can be subjected to. Tell-tale examples of the same are prison inmates, detainees, convicts and kidnapped pupils. This is one of the stressors for which we as humans have minimal adaptability and hence they produce enormous repercussions in behavioral, biochemical and physiological dynamics of the individual.

Such stressors can be studied in rodent models relatively easily by methods like: restraining the animal in a tube (cylindrical/semi-cylindrical) for 2-3 hours, or by gently restraining its limbs by packing them in a cloth for 2-3 hours or even by restraining its neck by the application of a wire. These experiments can be done both for a one-time stressor response evaluation (acute restraint stress) or by subjecting the animal to the aforementioned tests for 1-2 weeks and then analyzing for chronic restraint stress.[38]

As already discussed, due to low adaptation for this type of stressor, the animal shall demonstrate a heightened anxious/depressive behavior in standard tests and this shall be applicable for its biochemical parameters of stress too.[39]

Electric foot-shock induced stress

This is a very unique stressor which can be applied on rodents whereby mild amperage (0.5-2 mA) electric shocks can be given for an acute duration of time (1-2 sec) and rodents, being sensitive to such a kind of stimulation do exhibit increased levels of anxiety which can be corroborated by standard tests and biochemical quantification.[40] The apparatus used here is a metal grid floor which is connected to a shock generator. This model can demonstrate the behavioral responses shown by organisms to hyper acute triggers/stressors in their environment.[41]

Social defeat stress

This model was proposed by Klaus Miczec. Here we introduce a single mouse (intruder) in the home cage of a resident male mouse (aggressor). During this test, behaviors related to confrontation of the intruder by the aggressor are studied. The time spent by an intruder in social defeat posture which is induced by the presence of an aggressor is computed through-out five trials by a blinded observer. Social defeat posture is identified by the following criteria: Immobility (intruder places all four paws on ground, and is oriented towards the aggressor), escape (whereby the intruder tries escaping from the aggressor), crouching (Intruder keeps all four paws on the ground, but isn't oriented towards the aggressor), or defensive upright stance (whereby the intruder stands in an erect fashion with its forepaws extended). This stressor protocol has great relevance in socio-historical context of human ethology (behavior) whereby people show tremendously raised anxiety and alarmed behavior when confronted by a stranger.[42] This rodent model can be studied both in acute instances of social defeat posturing as well as the behavioral traits exhibited by the intruder upon prolonged/repetitive exposure to the resident aggressor whereby adaptation and diminished levels of anxious behavior is expected. These findings can be further qualified by subjecting the respective animal models to Standard tests and biochemical quantification of markers of anxiety.[43]

Chronic unpredictable stress

This model has been widely used to induce persistent stress-related behavioral changes in rodents. It consists of randomly presenting different stressors to the rodents daily. This prevents the stress-adaptation process which is observed in other models of chronic stress. Here animals are exposed for 2-5 weeks to a wide range of stressors, like foot shocks, restraint stress, light-dark cycle reversal, unpleasant noises, changes in the home cage, heating (37°C) or cooling (4°C) of the home cage, etc. After being exposed to these stressors for several days, the animals exhibit an increased Hypothalamic-Pituitary-Adrenal axis

sensitivity and a reduction of responses to pleasant stimuli, however the exploratory behavior is unfazed.[44] This model has good face validity as it represents the stressors faced by human beings in everyday life more realistically. Also, it has an excellent predictive validity, since repeated treatment with antidepressants like fluoxetine, desipramine or imipramine reverses the behavioral effects induced by this model.[45]

OTHER MODELS OF ANXIETY

Grooming analysis algorithm

Stress has long been known to affect grooming in rodent species, altering both its activity measures and behavioural microstructure. The rat grooming analysis algorithm (based on ethological analysis of incorrect transitions contrary to the cephalocaudal rule, interrupted grooming activity and the assessment of the regional distribution of grooming) and applied this algorithm to the light-exposed (high stress) and dark-exposed (low stress) groups of rats.[6] Various results suggest that this method can be useful tool in neurobehavioural stress research including modelling stress-evoked states, psychopharmacological or behavioural neurogenetics research in rats.[46]

Escape behavior induced by electrical/chemical stimulation of Dorsal Portions of the Periaqueductal Grey Matter (Dpag) as a model of panic disorder

PD is chronic and psychiatric disabling disorder featured by unexpected and recurrent attacks and about 5% people of worldwide are affected by this. PD patients psychosocial impairment and have a high risk of psychiatric comorbidities and suicide. The periaqueductal grey matter (PAG) is a midbrain structure that, with other functions, integrates defensive behavior. In humans, electrical stimulation of this evokes strong feelings of fear, impending death, non-localized pain, and marked autonomic changes. Given the striking similarities between autonomic and behavioral effects of dPAG stimulation and symptoms of panic attacks, it has been suggested that this structure is involved in the genesis of PD in humans and that stimulation of this midbrain area in animals can model panic attacks.[47] Stimulation of dPAG is usually performed in a circular arena (40 cm in diameter) with 40 cm-high walls made of transparent Plexiglas. For chemical stimulation, direct injection of an N-methyl-D-aspartate (NMDA) agonist or GABAergic antagonist induces defensive behaviors. For electrical stimulation, a brain electrode is connected to the stimulator by means of an electromechanical swivel and a flexible cable, allowing ample movement of the animal inside the experimental cage. The current is generated by a sine-wave stimulator and monitored on the screen of an oscilloscope. After stimulation of t dPAG, a vigorous reaction is observed, with freezing response, piloerection, miosis, vertical jumps, and a strong flight reactions represented by increase in locomotion and average speed.[48]

CONCLUSION

The relationship between the anxiety models and the clinical profile of anxiety disorders is quite low. The above listed models for anxiety are useful to understand the activity of various pharmacological compounds and their mechanism of action. But these models have their own advantages and disadvantages like that they are susceptible to environmental changes. It is the responsibility of the researcher to select the appropriate model to investigate newer compound or to understand the pathways involved in anxiety. The future of new drugs in anxiety disorder relies on the development of new models based on individual vulnerability to anxiety and genetic or epigenetic factors.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

REFERENCES

1. Craske MG, Stein MB, Eley TC, Milad MR, Holmes A, Rapee RM, Wittchen HU. Anxiety disorders. Nat Rev Dis Primers. 2017;3:17024.
2. Campos AC, Fogaça MV, Aguiar DC, Guimarães FS. Animal models of anxiety disorders and stress. Braz J Psychiatry. 2013;35(2):S101-11.
3. Montgomery KC. The relation between fear induced by novel stimulation and exploratory behavior. J Comp Physiol Psychol. 1955;48(4):254-60.
4. Hogg S. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol Biochem Behav. 1996;54(1):21-30.
5. Hart PC, Bergner CL, Smolinsky AN, Dufour BD, Egan RJ, LaPorte JL, et al. Experimental Models of Anxiety for Drug Discovery and Brain Research. In: Proetzel G, Wiles MV, eds. Mouse Models for Drug Discovery. New York, NY: Springer New York; 2016: 271-291.
6. Harro J. Animals, anxiety, and anxiety disorders: How to measure anxiety in rodents and why. Behav Brain Res. 2018;352:81-93.
7. Pinheiro SH, Zangrossi H, Del-Ben CM, Graeff FG. Elevated mazes as animal models of anxiety: effects of serotonergic agents. An Acad Bras Cienc. 2007;79(1):71-85.
8. Graeff FG, Netto CF, Zangrossi H. The elevated T-maze as an experimental model of anxiety. Neurosci Biobehav Rev. 1998;23(2):237-46.
9. Kulkarni SK, Singh K, Bishnoi M. Elevated zero maze: a paradigm to evaluate antianxiety effects of drugs. Methods Find Exp Clin Pharmacol. 2007;29(5):343-8.
10. Bell R, Duke AA, Gilmore PE, Page D, Bègue L. Anxiolytic-like effects observed in rats exposed to the elevated zero-maze following treatment with 5-HT2/5-HT3/5-HT4 ligands. Sci Rep. 2014;4:3881.
11. Bell R, Duke AA, Gilmore PE, Page D, Bègue L. Anxiolytic-like effects observed in rats exposed to the

elevated zero-maze following treatment with 5-HT2/5-HT3/5-HT4 ligands. Sci Rep. 2014;4:3881.
12. Crawley J, Goodwin FK. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav. 1980;13(2):167-70.
13. Hall CS. Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. J Comp Psychol. 1934;18(3):385-403.
14. Crawley JN. Exploratory behavior models of anxiety in mice. Neurosci Biobehav Rev. 1985;9(1):37-44.
15. Brown GR, Nemes C. The exploratory behaviour of rats in the hole-board apparatus: Is head-dipping a valid measure of neophilia? Behav Processes. 2008;78(3):442-8.
16. Toth I, Neumann ID. Animal models of social avoidance and social fear. Cell Tissue Res. 2013;354(1):107-18.
17. Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav Brain Res. 1988;31(1):47-59.
18. Klebaur JE, Bardo MT. The effects of anxiolytic drugs on novelty-induced place preference. Behav Brain Res. 1999;101(1):51-7.
19. Dixit PV, Sahu R, Mishra DK. Marble-burying behavior test as a murine model of compulsive-like behavior. J Pharmacol Toxicol Methods. 2020;102:106676.
20. Himanshu, Dharmila, Sarkar D, Nutan. A Review of Behavioral Tests to Evaluate Different Types of Anxiety and Anti-anxiety Effects. Clin Psychopharmacol Neurosci. 2020;18(3):341-51.
21. Li X. Using the conditioned fear stress (CFS) animal model to understand the neurobiological mechanisms and pharmacological treatment of anxiety. Shanghai Arch Psychiatry. 2012;24(5):241-9.
22. Shoji H, Takao K, Hattori S, Miyakawa T. Contextual and cued fear conditioning test using a video analyzing system in mice. J Vis Exp. 2014;(85):50871.
23. Millan MJ, Brocco M. The Vogel conflict test: procedural aspects, gamma-aminobutyric acid, glutamate and monoamines. Eur J Pharmacol. 2003;463(1-3):67-96.
24. Babbini M, Gaiardi M, Bartoletti M. Benzodiazepine effects upon Geller-Seifter conflict test in rats: analysis of individual variability. Pharmacol Biochem Behav. 1982;17(1):43-8.
25. Finnell JE, Lombard CM, Padi AR, Moffitt CM, Wilson LB, Wood CS, et al. Physical versus psychological social stress in male rats reveals distinct cardiovascular, inflammatory and behavioral consequences. PLoS One. 2017;12(2):e0172868.
26. Finnell JE, Lombard CM, Padi AR, Moffitt CM, Wilson LB, Wood CS, et al. Physical versus psychological social stress in male rats reveals distinct cardiovascular, inflammatory and behavioral consequences. PLoS One. 2017;12(2):e0172868.
27. Cyr S, Abuaish S, Spinieli RL, McGowan PO. Maternal Predator Odor Exposure in Mice Programs Adult Offspring Social Behavior and Increases Stress-Induced Behaviors in Semi-Naturalistic and Commonly-Used Laboratory Tasks. Front Behav Neurosci. 2018;12:136.
28. Zimmerberg B, Rosenthal AJ, Stark AC. Neonatal social isolation alters both maternal and pup behaviors in rats. Dev Psychobiol. 2003;42(1):52-63.
29. Lai MC, Yang SN, Huang LT. Neonatal isolation enhances anxiety-like behavior following early-life seizure in rats. Pediatr Neonatol. 2008;49(2):19-25.
30. Lai MC, Yang SN, Huang LT. Neonatal isolation enhances anxiety-like behavior following early-life seizure in rats. Pediatr Neonatol. 2008;49(2):19-25.
31. Landgraf D, Long JE, Proulx CD, Barandas R, Malinow R, Welsh DK. Genetic Disruption of Circadian Rhythms in the Suprachiasmatic Nucleus Causes Helplessness, Behavioral Despair, and Anxiety-like Behavior in Mice. Biol Psychiatry. 2016;80(11):827-35.
32. Westman JC, Walters JR. Noise and stress: a comprehensive approach. Environ Health Perspect. 1981;41:291-309.
33. Jensen K, Hahn NE, Palme R, Saxton K, Francis DD. Vacuum-cleaner noise and acute stress responses in female C57BL/6 mice (Mus musculus). J Am Assoc Lab Anim Sci. 2010;49(3):300-6.
34. Oka T. Stress-induced hyperthermia and hypothermia. Handb Clin Neurol. 2018;157:599-621.
35. Karp CL. Unstressing intemperate models: how cold stress undermines mouse modeling. J Exp Med. 2012;209(6):1069-74.
36. Veening JG, Bouwknecht JA, Joosten HJ, Dederen PJ, Zethof TJ, Groenink L, et al. Stress-induced hyperthermia in the mouse: c-fos expression, corticosterone and temperature changes. Prog Neuropsychopharmacol Biol Psychiatry. 2004;28(4):699-707.
37. Vinkers CH, Oorschot RV, Olivier B, Groenink L. Stress-Induced Hyperthermia in the Mouse. Springer. 2009;42:139-52.
38. Paré WP, Glavin GB. Restraint stress in biomedical research: a review. Neurosci Biobehav Rev. 1986;10(3):339-70.
39. Hwang DJ, Kwon KC, Hwang DY, Seo MS, Kim KS, Jung YS, et al. Comparative analysis of restraint stress-induced depressive-like phenotypes in C57BL/6N mice derived from three different sources. Lab Anim Res. 2020;36:29.
40. Chida Y, Sudo N, Motomura Y, Kubo C. Electric foot-shock stress drives TNF-alpha production in the liver of IL-6-deficient mice. Neuroimmunomodulation. 2004;11(6):419-24.
41. Bali A, Jaggi AS. Investigations in foot shock stress of variable intensity in mice: Adaptation and role of angiotensin II. Eur J Pharmacol. 2015;761:86-94.
42. Doeselaar L, Yang H, Bordes J, Brix L, Engelhardt C, Tang F, Schmidt MV. Chronic social defeat stress in female mice leads to sex-specific behavioral and neuroendocrine effects. Stress. 2021;24(2):168-80.
43. Yoshida K, Drew MR, Kono A, Mimura M, Takata N, Tanaka KF. Chronic social defeat stress impairs goal-

directed behavior through dysregulation of ventral hippocampal activity in male mice. Neuropsychopharmacology. 2021;46(9):1606-16.
44. Monteiro S, Roque S, de Sá-Calçada D, Sousa N, Correia-Neves M, Cerqueira JJ. An efficient chronic unpredictable stress protocol to induce stress-related responses in C57BL/6 mice. Front Psychiatry. 2015;6:6.
45. Nollet M. Models of Depression: Unpredictable Chronic Mild Stress in Mice. Curr Protoc. 2021;1(8):e208.
46. Kalueff AV, Tuohimaa P. The grooming analysis algorithm discriminates between different levels of anxiety in rats: potential utility for neurobehavioural stress research. J Neurosci Methods. 2005;143(2):169-77.
47. Camplesi M, Bortoli VC, Soares VP, Nogueira RL, Zangrossi H. Dorsal periaqueductal gray stimulation facilitates anxiety-, but not panic-related, defensive responses in rats tested in the elevated T-maze. Braz J Med Biol Res. 2012;45(11):1025-30.
48. Kim EJ, Horovitz O, Pellman BA, Tan LM, Li Q, Richter-Levin G, et al. Dorsal periaqueductal gray-amygdala pathway conveys both innate and learned fear responses in rats. Proc Natl Acad Sci U S A. 2013;110(36):14795-800.

Cite this article as: Desingu R, Sadasivam B, Kalra SS, Lakhawat B. Animal models of anxiety: a review. Int J Basic Clin Pharmacol 2023;12:134-41.