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REVIEW ARTICLE RESPONSE BASED ANIMAL ANXIETY MODELS

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ABSTRACT

An animal model is an imitation that resembles human being anatomically and physiologically. For the study of the pathophysiology of a disease and drugs used for the treatment of those diseases animal models are employed. The use of animal models for the study of the biological basis of the psychiatric disorders is well known. The exact mechanism of action can be screened out by the animal models. Anxiety is a normal human emotion but is of concern in pathological state. Anxiety models were initiated with the use of rats to screen the various anti-anxiety drugs and now being having a vast success rate with use of mice too for the study. Anxiety animal models are categorized as the conditioned and unconditioned response based models. Animal models are validated by the three types of validities- *Face validity* in this the responses shown by the model will be similar to that of human being as model is phenotypically similar, *Predictive validity* animal models are sensitive to clinically effective pharmacological agents, *Construct validity* shows the similarity between the human behaviour and hypothetical animal models. The review focuses on the different types of anxiety models based on the conditioned and unconditioned responses.

INTRODUCTION

Anxiety refers to the intrinsic, adaptive mechanism that tends the body to respond to existent or illusory dangers and problems. However, in case anxiety becomes pathological it shows severe emotional and physical consequences[1]. A condition to feel worried about an unknown danger, apprehension, and nervousness or discomfort whose decision is doubtful can be termed as anxiety[2].It is thought to be amongst the most widespread psychiatric syndromes touching 10 to 30% of the common population of world effecting feeling and cognition. It also demonstrates co-morbidity with depression[3].The most well known symptoms include restlessness, insomnia, fatigue, headache, impaired concentration, confusion, irritability. Exposure to certain substances and drugs can trigger anxiety symptoms which include – caffeine, cannabis, cocaine and drugs like salbutamol, insulin, thyroid hormones etc[4]. Benzodiazepines are the class of medications that are recommended to reduce the symptoms of anxiety. The pharmacological action of benzodiazepines is through high affinity binding sites on a complex composed of GABA-A and BZD receptor coupled with a chloride channel

,stimulation of GABA-A receptor results in amplified chloride conductance and hence response elicited[5]. These drugs are being evaluated through the various animal models of the anxiety; present review focuses on the different types of animal models used for the evaluation of the anti-anxiety drugs. These different models help in assessing the various drugs according to the behaviors elicited by the subjects.

ANIMAL MODELS OF ANXIETY

Animal models are biological imitations developed for the purpose of studying the physiological changes and responses elicited by the human beings in response to certain conditions and drugs; the acceptance of the model is limited to the similarity with humans. Animal models are widely employed for the study of the neurobiological basis of the psychiatric disorders. Although there is no such evidence regarding the success of these models for the psychological changes, we still only assume on the theoretical basis that the changes that are occurring in an model are similar to that of the human being. Even though animal models have emerged as an intensive tool for the study of the various drugs and they can also depict symptoms concerned with a specific disorder. Presently, animal models have these three types of the validities: *Face validity*, phenotypically similar models are being employed and it is considered that the behavior and psychological responses observed in the model will be resemblance of those observed in humans .Although, there is a significant difference between the behaviors of the rodents and that of humans ,a major one being verbal characteristic which is absent in animals.

• *Predictive validity* clinically effective pharmacological agent's sensitive model should be employed and on the other hand anxiogenic compounds should bring forth opposite effects, whereas agents not effective clinically shows any effect.

• The construction validity criterion refers to the similarity between the theoretical logic that underlies the animal model and human behavior. This requires that the etiology of the behavior and the biological factors underlying the disorder be similar in animals and in humans. Researchers often do not specify whether they are looking for a correlation model (for example, predictive validity, a model that is selectively sensitive to therapeutic agents), an isomorphic model (apparent validity, a model that implies that the behavioral response in humans and animals) is identical) or a homologous model (the true validity of the construct, a model that involves the cause of the behavioral response in the animal is sufficient to elicit the same response in humans). A process and an event can be qualified as behavior. All processes of the underlying organic system with respect to exposure to the external physical and social environment constitute the observable behavior[6].As mentioned above, many animal models are derived from the discovery of benzodiazepenes and non-benzodiazepines anxiolytics, for example, buspirone, have been shown to be inactive in some anxiety tests. It has become clear that anxiety is not a unitary disease, but a complex phenomenon that probably involves many different neurochemical systems with diverse etiological origins and can be divided into several forms, including state and trait anxiety. Animals cannot model all aspects of human anxiety, but animal studies allow a detailed study of neurobiological and psychological processes in states in which fear could be inferred, such as responses to acute attacks. The clinical acceptance of the heterogeneity of anxiety disorder suggests that there are different neurobiological substrates for each one, and therefore it is necessary to examine if different tests in animals can reflect these differences. Assigning specific anxiety tests for particular anxiety disorders is an extremely difficult task. Therefore, several animal

models may be more appropriate for one type of anxiety disorder than another because it is inappropriate to assume that either model can be used to detect compounds for a disease transmitted through multiple and diverse mechanisms[7].

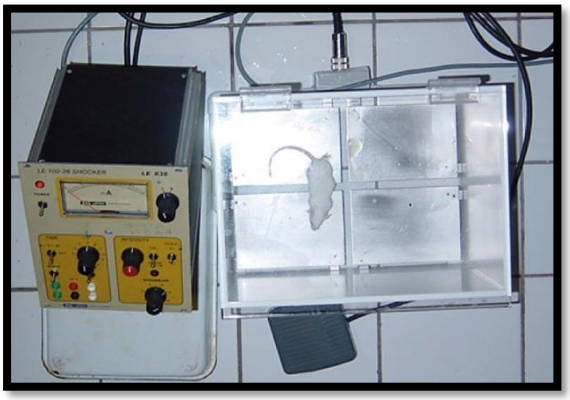
CLASSIFICATION OF THE MODELS

The classification of animal models of anxiety according to the nature of the aversive stimulus and of the response elicited, suggesting that the neuronal control of anxiety may differ according to whether the interpretation of an aversive signal is innate or learned, and whether it elicits a response or, conversely, inhibits an ongoing, rewarded behavior. The two main sub divisions of the animal models of anxiety are- the very first classification termed as conditioned response involves animal exposure to stressful and painful trial(e.g., electric foot shock contact); the second includes ethologically based paradigms and involves the animal's spontaneous or natural reactions (e.g., flight, avoidance, freezing) to stress stimuli that do not explicitly involve pain or discomfort (e.g., exposure to a novel highly illuminated test chamber or to a predator)[8]. Ethologically based animal models of fear and anxiety attempt to approximate the natural conditions under which such emotional states are elicited. To stimulate fear and anxiety non aversive stimuli are being used, ethological indication is thought to reduce the potential baffling effects of motivational or perceptual states that result from interference with hungry / thirsty or nociceptive learning / memory mechanisms . Experimental interventions compared with conditioned models, ethological tests seem better qualified to be analogous to human anxiety Ethological models, however, have individual differences and different levels of behavior, however, ethological stimuli are of a different nature .When an electric shock is associated with a neutral stimulus, a conditioned fear occurs in the animal, the presentations of later stimuli interrupt the current behavior and produce avoidance or defense. The previous training of the subjects to achieve specific response levels decreases the individual variability. The automated evaluation of the parameters studied, combined with a rigorous control and the methodological manipulation of the experimental variables, and also facilitates the use of conditioned models. However, these tests require a lot of time for your subjects. The need for motivation (deprivation of food and / or water) and the participation of painful stimuli or events often confuse the results, leading to other possible interpretations. In addition, the influence of past experience with drugs and, often, low baseline response rates are additional challenges in conflict paradigms[9].

CONDITIONED RESPONSES

Classical conditioning experiments engross an associative learning process in which a neutral conditional stimulus (CS) is frequently exposed with an unconditional stimulus (US). After the constant pairings, the CS presentation alone will stimulate affective responses depicted as a conditional emotional reaction in the subjects [10].

Figure 1 - Four- Plate Test



FOUR PLATE TEST

In the four plate test method, we generally test or examine the repression of innate ongoing behavior or we can say that the examining of novel surroundings of the mouse. It consists of floor which is made of four identical rectangular metal plates. It is based on the principle, that when the mouse crosses the quadrant its behavior is suppressed by the liberation of mild electric foot shock. This mild shock, when crossing the floor from one place to another, eliciting a clear flight reaction from the animal[11]. Some synthetic drugs like BZDs improve or increase the number of crossing accepted by the animal[12].Before any conclusion can be drawn for a drug tried in this test, it is necessary to verify that this drug has no analgesic effects. Please keep in mind when utilizing hot plate apparatus employ morphine as a control compound. This model is generally used in other laboratories making it difficult to make inter-laboratory comparison. It was reported that a single prior undrugged exposure to the FPT reduces punished responding on retest at intervals ranging from 24 hours to 42 days[13]. In addition, preceding experience attenuates the anxiolytic comeback to benzodiazepines, diazepam and lorazepam. Similar to results observed in EPM and L/D. On the other hand, this test is also very helpful to explain mechanism of anti-anxiety drugs using antagonist at level of 5-HT, Gama-amino butyric acid and corticotrophin releasing factor. In laboratories, the FPT is used on large scale for detection of the anxiolytic activity of new developed compounds [14].

VOGEL THIRSTY RAT CONFLICT TEST

To check the anxiolytic activity, a easy and consistent conflict procedure is described. Shocks are administered to thirty rats by licking the water. Rats are deprived of water for 48 hours. Two hours before the test, each rat is placed in a transparent Plexiglas box (38 × 38 cm) with a black Plexiglas compartment (10 ×10.5 cm) attached to a wall and an opening of the large box to the small compartment. The whole device has a floor of Stainless steel. A water bottle with a metal tube is placed outside the small compartment, so that the tube extends into the box at a height of 3 cm above the grid. The rats are bursting with a relatively constant speed of 7 licks per second. A watering circuit is connected between the watering tube and the ground of the apparatus, so that the rat completes the circuit each time it licks the tube. The shock is delivered to the feet of the animal by changing the connections between the trough tube and the core grids into a shock device that applies an unresolved shock between the trench tube and the ground of the trench[15].The rat is placed in the apparatus and is allowed to find the tube to drink and to complete 20 licks before the application of the shock (available in the tube for 2 s). The rat controls the duration of the shock by withdrawing from the tube. A 3-minute timer will automatically start at the end of the first download. During the 3 minute period, the shocks are administered every twenty times. The quantity of downloads administered during the 3-minute session is recorded for each animal. The number of collisions sustained after treatment is compared to that of untreated animals[16].

Figure 2 -Vogel Thirsty Rat Conflict

GELLER TYPE CONFLICT TEST

Hunger is the underlying principle of the test and it is induced in animals by depriving them for food. A prior training is given to the animals for the experiment under MULT FR20/FR20-punishment

schedule of food fortification[17]. The program consists of four pairs of a discontinuous safe period; the animals' lever pressing is armored by food pellets at FR20 lacking electric shock. During the alarm period, which is indicated by a warning stimulus (tonic signal:80 Hz, 90 dB), every 20th lever press is punished using an electric shock (50–90 V, ca 0.3 mA, 50 Hz AC, duration 0.3 s). The reaction rate of the animal is recorded during the safe as well as alarm period[18].

ACOUSTIC STARTLE RESPONSE

The acoustic response of surprise is a uncomplicated behavior that occurs unsurprisingly in mammals and is affected by various treatments. The test response can be used to resolve the sites and mechanisms of action of the drug. The acoustic response consists of a sequence of fast movements that begin in the head and connect the contraction and extension of major muscle groups in response to auditory stimuli with a rapid onset of time[19]. Responses are ranked in amplitude relative to stimulus intensity and may show habituation and awareness. The assay has been modified in several ways, for example inhibition by pre-propulsion or fear-induced potentiation has studied the interruption of the inhibition of surprise response by the pre-pulse in the rat by the mediated by 5-HT2A receptors[20]. The authors suggested that serotonergic substrates studies of prepulse inhibition could provide a model of the possible serotoninergic role in sensorimotor activation abnormalities in schizophrenic patients and in patients with obsessive-compulsive disorder. It began that the amplitude of the acoustic response in the rat was increased by the high levels of illumination[21].

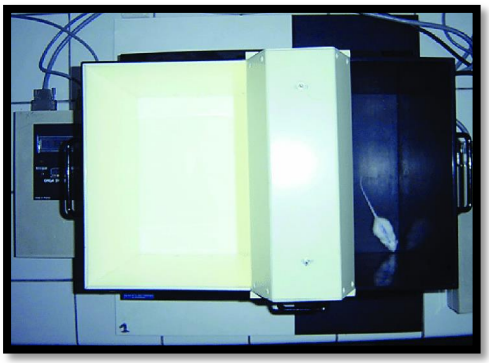
UNCONDITIONED RESPONSES

The unconditioned response study exposes the subject to the different forms of the external threats and helps in extension of our logics and stimulations, of what found in nature (intrinsic fear/avoidance). These models are anticipated to have a elevated logistic validity, creating a more detailed depiction of the behavioral changes induced by the tests. The basic principle of most of these models is the set of behavioral responses induced by exposure to a new environment, which concurrently evokes fear and curiosity, creating a characteristic approach avoidance conflict[22].

THE LIGHT/DARK BOX

A new distinctive model of anxiety is Light/Dark exploration test. Exploratory behavior of rodents is depicted by the apparatus and rodents are affected by the bright light propagated in the box, the two paradigms are basic principle of the apparatus. The two organized compartments that differ from each other in size (2:1), color (white: black) and illumination (dim light and intense light) is being explored by the rodent.

Figure 3 - Light Dark Box



Consequently, dark area is preferred by the rat. Evident dread of remaining in or going into the bright illuminated area is established when treated with an anti-anxiety drug. Initially the test was performed with the mice but later on trials on the use of rats with some crucial modifications was also succeeded for evaluating anti-anxiety agents. The prototype model was subjected to a variation by introducing a tunnel type runway between the two compartments and size of the compartments was also enlarged. The time spent /behavior elicited by the rodent is some of the modified index included in the study[23]. Five main parameters are now available to assess the

anxiolytic profile of drug treatment: the latency time for the first passage from the light compartment to the dark one, the number of transitions between the two compartments, the movement in each compartment, and the time spent in each compartment. Sometimes rearing and grooming are measured. Benzodiazepens decrease the number of attempts at entry in the aversive area as mice pass directly into the lit compartment without hesitation, a profile suggested of being indicative of anxiolytic-like activity[24]. A parameter suggested as an index of the effect of anxiogenics is the "leaning out" or "peeking out" of the dark chamber by the mouse, somewhere a decrease in the rate of leaning out appears to be a constant effect of standard anxiety-inducing drugs. However, these behaviors are invariably ignored in favor of a simple spatiotemporal index, and the measurement found to be most consistent and useful for assessing anxiolytic-like activity action is the time spent in the lit compartment, as this parameter provides the mainly consistent dose-effect responses with different compounds. There are a number of non genetic, non pharmacological manipulations that lead to modulation of the general stress levels of animals, which when performed before testing have profound effects on behavior in the L/D model. Prior exposure of mice to the EPM eliminates the anxiolytic response to diazepam in the L/D paradigm, whereas tail suspension acute stress immediately before the test can increase the sensitivity to anxiolytic-like responses[25]. Forced swimming suppresses general behavioral activity and increases the disinhibition effect of diazepam in both compartments, whereas foot shocks given immediately before the test significantly reduced the activity in the dark compartment and did not affect the behavior in the light compartment. Exposure of mice to predator odor (mimicked by 2,5-dihydro-2,4,5-trimethylthiazoline) or control odor (mimicked by butyric acid) induced anxiety in the L/D test relative to saline treated mice. Mice exposed to either butyric acid took longer to re-enter the light section of the apparatus and also spent less time in the light division relative to mice exposed to saline. Data indicate that prior test experience seriously compromises the anxiolytic efficacy of chloradiazepoxide in the mouse L/D test without significantly altering behavioral baselines. The choice of strain and age of the animal is also an important factor. The light/dark box test is still useful for discovering new targets in the field of anxiety–related disorders[26].

OPEN FIELD TEST

The test involves the observation of the usual locomotor activity, exploratory behavior and anxiety measures at a time[27].The field of the test is constructed with the plain wood and comprises of the square area (60 × 60 × 35 cm). Square of (60 × 60 cm) encloses the overall floor; the surface has 16 squares of (15 × 15 cm). A 60w bulb placed at a height of 100cm illuminates the apparatus.

Figure 4- Open Field Test

Following the pre-study time period of 45minutes of drug administration rat is

placed in the center of the open field and study time of 5min following behavioral observation is conducted. Video recording helps to record the parameters to be observed for a period of 5 min and they are as follows- [28]:1) time duration of subject in center and number of enteries by it in center (2) border and corners of the field, (3) distance travelled confirmed by the

number of crossings, (4) rearing and (5) four paws touching the walls of the apparatus.

SOCIAL INTERACTION TEST

The social interaction test is constructed in order to fulfill the gap for evaluating anti-anxiety and anxiogenic effect simultaneously. In general, anti-anxiety

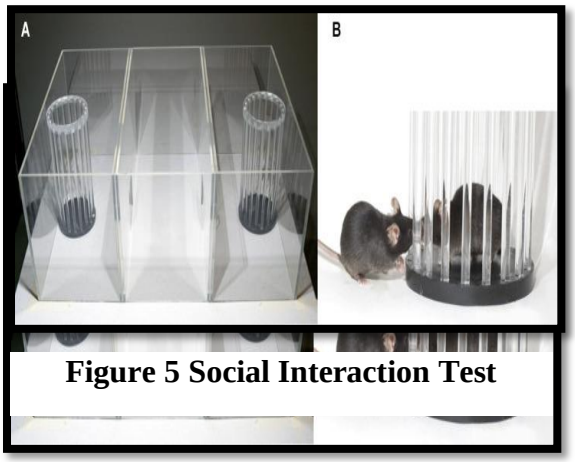


Figure 5 Social Interaction Test

effect is indicated through the increased social interaction; on the other hand decreased social interaction reflects anxiogenic effect. The test is the best advancement for knowing the neurobiological mechanisms involved in anxiety disorders. Overall 5-HT and DA levels in the rat brain are being elevated by the prevailed conditions in the test. Different kinds of the pharmacological agents used for the specified anxiety disorders can be evaluated effectively through the test. A black Plexiglas box test field is used, 60 × 60 × 35 cm, 9cm squares on the base are made with the lines of white tape respectively. A 380 lx intense light for the floor is used. Two different kinds of the circumstances are performed: high light, unfamiliar arena and high light, familiar arena. Day 1 of the test involves the selection of the animals on the basis of the weight and placing the animal with unfamiliar animal in group of 12 animals (6pairs). The animals were given the drug used and then returned to their home cages until testing. The pretreatment time is strictly followed up, unknown members in the each group are placed at the corners of the field and social interaction behaviour is being observed and also locomotors activity is noted down for a period of about 10minutes. The apparatus is cleaned up at the end of the testing time duration. Different kinds of the parameters that are observed in social interaction are sniffing time, grooming altogether, nearby lying and crawling over each other[29]. Aggression is also shown by the animals through kicking each other, biting, jumping and even boxing a times: these parameters are also noted down in the test and evaluated through different kinds of agents other than for anxiety disorders[30]. Subsequent completion of the first test, rats are returned to their home cages. Rats are familiarized with the apparatus on the 2 and 3 day by placing them in the box without drug individually. On the fourth day, the same pairs of rats are once again placed in the test field for a period of 10 min and the same test process is conceded out [31].

STAIRCASE TEST

The test is being used for screening anti-anxiety activity in animal. The apparatus works on the basic principle of exploration and locomotor activity which is provoked by the apparatus itself. Anxiety behavior is reflected by the rearing behavior of the mice. The apparatus is composed of the five indistinguishable steps 2.5cm×10cm×7.5cm. Along the whole length of staircase the internal height of the wall is steady. One animal at a time is used and only once in apparatus. The drug is given 1h or 30min before test. On the floor of the box animal is placed with its back to the staircase. For a period of 3minutes number of steps climbed and number of rearings is counted. When all the four paws of the animal are placed on a step than the animal is considered to be climbed. After each trial the apparatus is cleaned with (10% ethanol) to remove the unwanted odour and waste products. The average number of steps and rearings of control group are taken 100% and the values of treated animal are expressed as percentage[32].

CONCLUSION

The animal models of anxiety play a crucial role in validating the various kinds of human responses. The behavior tests developed through infuriating various human conditions is helpful for better understanding human behavior in anxiety and to get through the exact mechanism of actions of drugs. Nevertheless, the various models developed are sensitive to pharmacology of benzodiazepines and are validated through them only which give rise to enormous need to develop models that work on other systems too and are helpful in validating pharmacology of other drugs also rather than restricting to benzodiazepines use only. Faced with the lack of reproducibility and susceptibility to non-BZD drugs in animal models of anxiety, tests have been subjected to plethora of variation and excess of parameters of measurement. Due to this potential anxiolytic compounds cannot be screened out with precision and accuracy because of insensitivity of model to other agents. Recently, reported that standardization of various models through the various mice genotypes gave rise to the sensitivity to other anti-anxiety drugs and to various kinds of behaviors earlier not detected. Standardization has offered a new way to qualitatively and quantitatively imitate all aspects of a measurement. It ensures that the results are now more extremely interpretable with more of the difficult experiments. The review focuses on the various types of the models used for screening the different anxiolytic drugs and they present a potential for the validation and effectiveness of the upcoming drugs in the anxiety.

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