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Research methods for animal studies of the anxiolytic drugs

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Abstract
One out of thirteen people are suffering from Anxiety disorders. So many drugs are available in market. But none of the drug is said to be safe. This lack of safety gives scope of future trials of newer anxiolytic drugs. This review focuses on the various Research Methods for studying the anxiolytic effect of drugs. The various methods are divided into two parts - Conditioned responses and Unconditioned responses. Under Conditioned responses, various methods are- Geller–Seifter conflict (GS), Vogel conflict, Four-plate test (FPT), Conditioned emotional response (CER), Conditioned taste aversion (CTA), Fear-potentiated startle, Defensive burying, Active/passive avoidance. Under Unconditioned responses, various methods are - Elevated plus maze (zero/T maze), Light/dark exploration (L/D), Social interaction, Open field, Ultrasonic vocalization (pain or separation), Fear/anxiety-defence test batteries, Staircase test, Holeboard and Predator.

Keywords: Anxiolytic, animal models of anxiety, Open field, Elevated Plus maze, Light/dark paradigm, four-plate test, Fear-potentiated startle, Vogel water-lick conflict test

1. Introduction
Anxiety is an unpleasant state of mind which affects normal routine of a person. In today's highly competitive world, one out of thirteen people are suffering from Anxiety disorders. Anxiety has manifold complications. It is one of the factors responsible for rise in blood pressure, loss of appetite, fighting with parents, peers, seniors; drug addiction, crime and many other social problems.

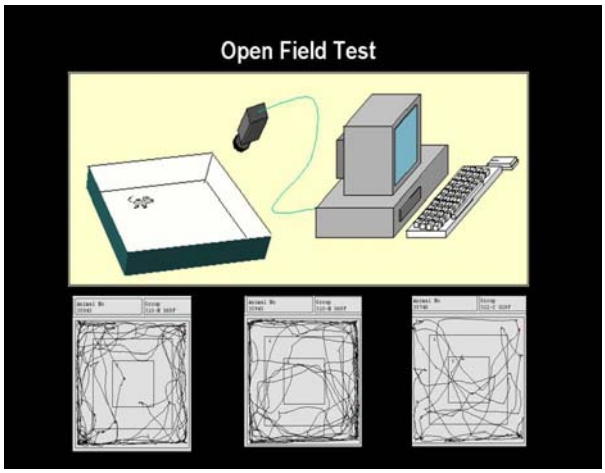
So many drugs are available in market. S But none of the drug is said to be safe. This lack of safety gives scope of future trials of newer anxiolytic drugs. This review focuses on the various Research Methods for studying the anxiolytic effect of drugs. The various methods are divided into two parts - Conditioned responses and Unconditioned responses. The broad classification of animal models of anxiety is as follows:

Table I: Classification of animal models of anxiety.

Conditioned responses	Unconditioned responses
1. Geller–Seifter conflict (GS)	1. Elevated plus maze (zero/T maze)
2. Vogel conflict	2. Light/dark exploration (L/D)
3. Four-plate test (FPT)	3. Social interaction
4. Conditioned emotional response (CER)	4. Open field
5. Conditioned taste aversion (CTA)	5. Ultrasonic vocalization (pain or separation)
6. Fear-potentiated startle	6. Fear/anxiety-defence test batteries
7. Defensive burying	7. Staircase test
8. Active/passive avoidance	8. Holeboard
9. Electrical brain stimulation (dPAG)	9. Predator

Out of these models, some of the simple and widely used methods are discussed below:

Open field
Open field test is developed by the scientist Hall. In this method, open field is divided into different squares. The animals are placed in an open field environment with walls at the periphery. Then the behavior of animal is studied. It is seen whether the animal remains in the centre of open field or stay on the periphery of the field without entering the centre. This is called thigmotaxis and said to be as anxiety behavior. Besides this the frequency of defecation and urination is also observed [1]. The number of squares visited in centre is divided by number of squares visited on periphery. The value of ratio will be less if the animal is more anxious.



Elevated plus maze (EPM)

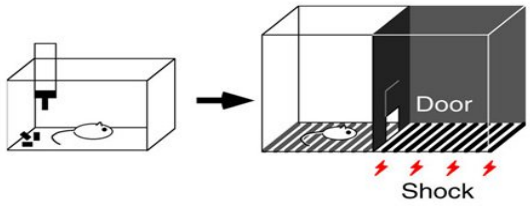
Elevated Plus Maze (EPM) is one of widely used behavioural and psychological model for research and screening of anxiolytic drugs [2–8]. As the name indicates, the Elevated Plus Maze is two open elevated arms of the same dimensions, crossing each other and form a 'plus'. There is a central square. The two arms are enclosed by walls. The maze is at certain height from the ground. These two arms provide mixed feelings of new, open as well as covered area with walls and elevation. The basis of Elevated Plus Maze is that the rodents generally avoid the open spaces. They generally behave differently for visiting or avoiding elevated open places.

Various animals are used in this method, like rats, guinea pigs, voles, hamsters and gerbils. The Elevated Plus Maze is modified into various shapes like elevated T-maze, zero maze and the unstable elevated exposed plus maze.

Light/dark paradigm

The light/dark (L/D) test is one of the widely used method for screening of anxiolytic drugs [9]. This method is developed by the scientist Crawley [10, 11]. In this model, there are two chambers- one is white and other one is dark or one part is well lighted and other part is dark i.e. without light. The principle of this method is that the rodents generally avoid areas with proper light. They try to spend more time in dark places. The control animal placed into the lighted area will rapidly move into the dark area. If a drug is anxiolytic, then the animal does not differentiate between light and dark area and freely move in both the areas.

Light Dark box.

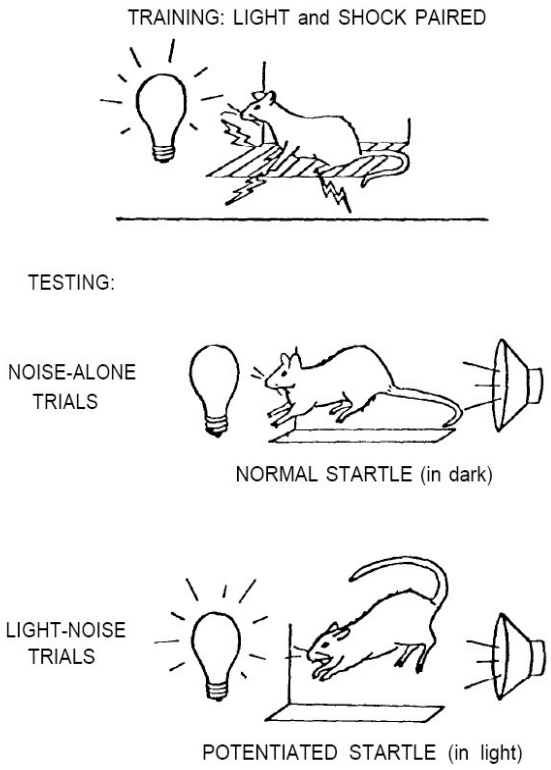


Four-plate test

The four-plate test is developed by the scientist Boissier *et al*. [12]. The apparatus is made up of four rectangular portion divided by two metal plates. The principle of four plate method is roaming nature of the animal. Whenever an animal is having anxiety, it will roam more frequently. In this method, every time the animal crosses from one rectangular portion to another, the metal plate will electrifies the whole floor. The animal will suffer an electric shock. When an anxiolytic drug is given to the animal, the animal will cross these rectangular area more frequently.

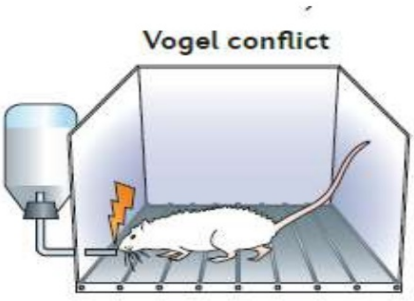
Fear-potentiated startle

Fear-potentiated startle method is developed by Brown *et al*. in 1951. This fear increasing method is comprised of two different steps [13]. In the First step, the animals are exposed to light, with an electric foot-shock. In the second step, animals are exposed to a loud sound. The animals startle in response to this unconditioned stimulus. When both the steps are repeated at the same time this startle response is increased. This increase in startle response can be found even after 1 month. When an Anxiolytic drug is given to the animal, the animal produce a dose-dependent decrease in the startle response.



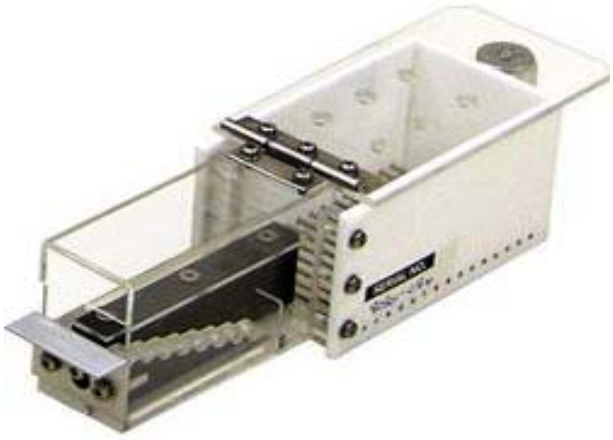
Vogel water-lick conflict test

Vogel water-lick conflict test is developed by Vogel *et al*. [14]. It is another widely used screening method for anxiolytic drugs. The primary animal used in this test is Rat. For this method a special cage is developed in such a way that the water feeding system is clubbed with an electric current. Whenever a thirsty animal drinks water it receives a mild electric shock [14]. So the animal will try to avoid drinking water. When an anxiolytic drug is given, the animal drinks more frequently despite more number of electric shocks [15].



The Staircase Method

As the name indicates, in the staircase test the animal is placed in an enclosed staircase. The staircase has only five steps. The animal is observed for three minutes. In these three minutes the animal has climbed how many steps and how many rearings it made. In some cases, the anxiolytic drugs did not reduce the number of steps climbed but reduce rearing. Many non-anxiolytic substances produced a decrease in both climbing and rearings. Some anticonvulsant increases step climbing but reduced rearing. The staircase test is a simple and fast method of testing anxiolytics [16].



2. Conclusion

Anxiety is fastly emerging as a social problem due to cut throat competition in today's world. Although a variety of Anxiolytic drugs are available, but all of these drugs suffer from one or other serious side effects. These lacuna offers scope for developing newer anxiolytic drugs. Although a wide variety of screening methods for Anxiolytics are available, but this review article tries to give a sneak review of some of the widely used screening methods. The researcher can pick any of the method which he finds himself comfortable with for screening Anxiolytics.

3. References

1. Hall CS. Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. J Comp Psychol. 1934; 18:385-403.
2. Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl.) 1987; 92180-185.
3. Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci. Methods. 1985; 14:149-167.
4. Jones N, King SM. Influence of circadian phase and test illumination on pre-clinical models of anxiety. Physiol. Behav. 2001; 72:99-106.
5. Belzung C, Griebel G. Measuring normal and pathological anxiety-like behaviour in mice: a review. Behav. Brain Res. 2001; 125:141-149.
6. Bourin M. Animal models of anxiety: are they suitable for predicting drug action in humans? Pol. J Pharmacol. 1997; 49:79-84.
7. File SE. Factors controlling measures of anxiety and responses to novelty in the mouse. Behav. Brain Res. 2001; 125:151-157.
8. Holmes A. Targeted gene mutation approaches to the study of anxiety-like behavior in mice. Neurosci. Biobehav. Rev, 2001; 25:261-273.
9. Bourin M, Hascoet M. The mouse light/dark box test. Eur. J Pharmacol. 2003; 463:55-65.
10. Crawley JN. Neuropharmacologic specificity of a simple animal model for the behavioral actions of benzodiazepines. Pharmacol. Biochem. Behav, 1981; 15:695-699.
11. Costall B, Jones BJ, Kelly ME, Naylor RJ, Tomkins DM. Exploration of mice in a black and white test box: validation as a model of anxiety. Pharmacol. Biochem. Behav. 1989; 32:777-785.

12. Boissier JR, Simon P, Aron C. A new method for rapid screening of minor tranquillizers in mice. Eur. J Pharmacol. 1968; 4:145-151.
13. Brown JS, Kalish HI, Farber IE. Conditioned fear as revealed by magnitude of startle response to an auditory stimulus. J Exp Psychol. 1951; 41:317-328.
14. Vogel JR, Beer B, Clody DE. A simple and reliable conflict procedure for testing anti-anxiety agents. Psychopharmacologia, 1971; 21:1-7.
15. Michel Bourin. Animal models of anxiety in mice. Fundamental & Clinical Pharmacology, 2007; 21:567-574.
16. Simiand J**,** Keane PE, Morre M. The staircase test in mice: A simple and efficient procedure for primary screening of anxiolytic agents. Psychopharmacology, 1984; 84:48-53.
17. The diagrams and pictures are taken from www. Google.com