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Pharmacology

A Short Review on Animal Models in Psychopharmacology

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Abstract **Review Article**

Psychopharmacological disorders are among the leading cause of non-fatal disease burden in India. One in seven Indians was affected by psychopharmacological disorders. Although psychopharmacological agents is used for the management of psychological disorders like anxiety, psychosis, depression and mania it may side effects such as tardative dyskinesia, weight gain, weight loss, muscle cramps, dysphoria, gastrointestinal upset, eye problems or problems with blood tests. In this present review we made an exposure regarding many screening methods on evaluation of psychopharmacological disorders like anxiety, depression, psychosis and mania in the aspects of preclinical studies. These methods are very useful to evaluate the psychopharmacological agents like anti-anxiety agents, anti-psychotics, anti-depressants and anti-manic agents.

Keywords: psychopharmacological disorders, preclinical studies, anti-anxiety agents, anti-psychotics, anti-depressants, anti-manic agents.

HISTORY:-[1, 2]

The past 50 years could easily be characterized as the age of psychopharmacology. From a cultural perspective, Prozac (fluoxetine; Eli Lilly and Company) has replaced Freud as shorthand for talking about what ails us. From an economic perspective, we spend more money on psychotropic drugs than on any other class of pharmaceuticals. From a clinical perspective, especially in the case of psychiatry, psychiatrists have become much more comfortable writing a prescription for an antidepressant than interpreting a patient's unconscious motivations. From a scientific perspective, psychotropic drugs have made possible fundamental insights into how the brain functions. Knowing her words would appear in America's most widely read national daily newspaper, USA Today, Tipper Gore no doubt intentionally simplified her explanation for her depression and how she fixed it. An advocate for better mental health care, Gore hoped her public confession would encourage others to seek treatment. Unpacking her quotation, she believes that her sadness was a disease like any other biological disease, rooted in biology with a specific pathophysiology. Importantly, it is a pathophysiology that is explained by a deficiency of serotonin. As a "clinical depression," her biological explanation suffices as a complete-enough description. Any other details about her life and relationships are extraneous to her real problem, her serotonin-depleted brain, which presupposes cure by a selective serotonin reuptake inhibitor (SSRI). Her explanation for her depression expresses a commonsense truth that we now take for granted and that we use simultaneously to explain as well as fix our distressed psyches. Our aim is to describe how this now almost-too-obvious fact became fact. The story is more complicated than we might think. Humans have used psychoactive substances since long before the advent of psychiatry, but the modern history of psychopharmacology begins in 1950.We chronicle four different eras. The first, the prehistory of modern psychopharmacology, spans from the mid-nineteenth century to 1950.The second, the golden age of psychopharmacology, begins with the synthesis of chlorpromazine in 1950 and ends in the mid-1960s. Researchers discovered all of our current psychotropic drug classes in this moment of unrivaled productivity. Paradoxically, psychiatrists embraced these new drugs with ambivalence, generally using them as adjuncts to what they saw as their more important psychosocial and psychotherapeutic activities. Spanning from the mid-1960s to the late 1980s, the third era marked startling and unprecedented changes for psychiatry, the science of the brain, the fate of patients, and the commonsense vision of disease and

therapeutics that we now take for granted. Psychotropic drugs played a central part in these dizzying changes. Our fourth era, the heyday of blockbuster antidepressants and antipsychotics, begins with the introduction of Prozac in 1988 into the US market. Over 30 years, the new drugs promised to radically transform the landscape of mental illness, but as the original patents expire, recent clinical trials have created doubt about their unalloyed benefits.

The earliest histories of any branch of science, just as the histories of anything else, tend to focus on chronologies of who discovered what and when and the recollections of eminent discoverers. This is very much the case in psychopharmacology at present, as the recent volumes on Discoveries in Pharmacology (Parnham and Bruinvels 1983) or Discoveries Biological Psychiatry (Ayd and Blackwell, 1970) illustrate. Undoubtedly important as this is, especially for younger generations of workers entering the field, such individual 'heroic' accounts implicitly relegate to roles of minimal importance other influences on development such as economic, or cultural factors. In some respects this is not surprising as medical historians have generally contrived to ignore the history of the drug business, seemingly in the belief that it is irrelevant to medical science, even though the production and use of drugs has increasingly underpinned medical business since the seventeenth century (Liebenau, 1987; Porter and Porter, 1989). Such neglect, however, seems particularly misplaced in psychopharmacology, which is a science that defines itself in terms of drug use and many of whose most eminent practitioners work in or in close association with pharmaceutical companies. This article seeks to offer some notes toward a future history that will surely locate the dynamic of advance in psychopharmacology other than solely with the insights and oversights of individual scientists one of the principal consequences of the introduction of antidepressants and neuroleptics was the development of the monoamine hypotheses of depression and the dopaminergic hypothesis of schizophrenia. However, far from these hypotheses being an unambiguous advance in the scientific understanding of mental illness, I have argued elsewhere (Healy, 1987a) that the monoamine hypotheses in particular were quite simplistic; that they accounted for less of the clinical data and were as unscientific as the psychodynamic hypotheses before them, in that they have been in practice, incapable of disproof. They did, however, legitimize research on the neural substrates of behaviour in a way the earlier hypotheses did not. They also provided opportunities for research, the benefits of which, as regards furthering our knowledge of brain processes and in bringing academic honours and research funding to contemporary neuroscientists and psychiatrists are quite clear. But the benefit in terms of increasing our knowledge of clinical disorders is much less clear. From the start, drug company research and promotional activity has had a dialectical relationship with academic interests.

INTRODUCTION:-[3]

Psychopharmacology is the study of how drugs affect mood, perception, thinking, or behavior. Drugs that achieve these effects by acting in the nervous system are called psychoactive drugs. The term psychopharmacology encompasses two large fields: psychology and pharmacology. Thus, psychopharmacology attempts to relate the actions and effects of drugs to psychological processes. A psychopharmacologist must have knowledge of the nervous system and how psychoactive drugs alter nervous system functioning. A psychopharmacologist can be a medical practitioner, like a psychiatrist, who specializes in prescribing psychoactive medication, or a scientist who studies psychoactive drugs. This approach defines the structure of this textbook. First, this book provides an overview of brain cells and structures. Second, it covers the basic principles of pharmacology. A psychopharmacologist must also characterize the effects of different types of drugs. We cover this after learning about the basic principles of pharmacology by considering the many different types of psychoactive drugs, beginning with recreational and abused drugs such as cocaine, marijuana, and LSD (Lysergic acid diethylamide) and ending with drugs for treating mental disorders such as depression, anxiety, and schizophrenia. Psychopharmacology is not the only term used to describe this field another term is behavioral pharmacology. Many consider behavioral pharmacology as synonymous with psychopharmacology, but others classify behavioral pharmacology as part of the subfield of psychology called behavior analysis. In this respect, drugs serve as behaviorally relevant stimuli just like other stimuli in behavior analytic models. Neuropsychopharmacology is another term for psychopharmacology. The neuro prefix represents the nervous system. Although the terms are similar, the Neuropsychopharmacology field has a particular emphasis on the nervous system actions of drugs.

In the United States, for example, consider the following:

- More than 100 million antidepressant drug prescriptions are written every year.
- More than 80 million anti-anxiety (i.e., anxiolytic), sedative, and hypnotic drugs are prescribed every year.
- More than 200 million pain-relieving drug prescriptions are written every year [Centers for Disease Control and Prevention (CDC), 2008].

When we add recreational drugs to the list, psychoactive drug prevalence in the United States increases further:

- ✓ More than 114 million adults consume alcohol on a regular basis (CDC, 2011).
- ✓ More than 25 million individuals use marijuana.
- ✓ More than 15 million individuals misuse a prescription drug.
- ✓ More than 70 million individuals use tobacco products (Substance Abuse and Mental Health Services Administration, 2010).

The World Health Organization (WHO) also reports high rates of psychoactive drug use internationally (WHO, 2012). For us, as students, teachers, researchers, and practitioners in psychology, to understand typical human behavior in the modern world, the sheer prevalence of drug use requires that we understand how drugs affect the way we think and function. The second reason for reading this text is that the statistics just presented show how nearly all of us are consumers of psychoactive substances; as consumers, we should know about the substances we ingest. Greater knowledge of psychoactive substances improves patient understanding of prescribed medical treatments and health implications of taking recreational and abused substances.

Third, you will come to understand how psychoactive substances provide important tools for understanding human behavior. The actions of antidepressant drugs led to understanding the roles that certain neurotransmitters and brain structures play in depression. Researchers use many experimental psychoactive drugs entirely as pharmacological tools for understanding brain function and behavior. Fourth, you will see how psychopharmacologist develops psychoactive treatments for psychological disorders. Rather, scientists trained in psychology test psychoactive drugs and determine their potential effectiveness for psychological disorders. This definition has challenges. The term administered excludes substances made naturally in the body. For example, the neurotransmitter dopamine is made in the nervous system and elicits important changes in nervous system functioning. However, hospital physicians may administer dopamine to a patient in order to elevate heart rate. In this context, dopamine is an administered substance that alters physiological functioning. Yet the same dopamine is made in the body—distinguishing the two leads us to call dopamine a drug when a practitioner administers it and call dopamine a neurotransmitter when the brain produces it.

The emphasis on physiological functions also has limitations. Certainly drugs produce changes in the body—but are food a drug? After all, food also produces physiological changes in the body Do drugs have a certain appearance? Drugs come in a variety of different forms, including pills, liquids, and powders. Most people consider nicotine a drug, although nicotine molecules reside within tar particles inhaled when smoking tobacco. Some teenagers may sniff certain types of glue, the vapors of which contain chemicals such as toluene. In this case, drugs also come in vapor form. Thus, although drug is a common term, we must not restrict our perception of a drug to a specific form or usage in psychopharmacology. Doing so risks excluding nonconforming substances that may have powerful effects for altering behavior.

PSYCHOACTIVE DRUGS:-

Psychoactive drugs broadly fall into two categories:

- ❖ Those intended for instrumental use.
- ❖ Those intended for recreational use.

The major distinction between these categories is a person's intent or motivation for using the substance. Instrumental drug use consists of using a drug to address a specific purpose.

For example, someone may take an antidepressant drug such as Prozac for the purpose of reducing depression. Further, most adults consume caffeinated beverages like coffee to help them wake up in the morning, another socially acceptable purpose. In psychopharmacology, instrumental use often occurs with therapeutic drugs—drugs used for treating disorders—for treating mental disorders such as depression and schizophrenia. Recreational drug use refers to using a drug entirely to experience its effects.

For example, recreational use of alcohol may consist of drinking alcohol purely to experience its intoxicating effects. Of course, we might describe alcohol use as instrumental if a person were only using it for another purpose such as relieving stress after a long day of work. Again, the intended use distinguishes instrumental use from recreational use. The term misuse applies to drugs that are intended for instrumental purposes but are instead used recreationally. For example, cough syrups that contain codeine or dextromethorphan are misused recreationally to achieve mind-altering effects such as euphoria or hallucinations.

Psychosis:-[4]

Psychosis refers to the mental illness where there is loss of contact with reality. Psychotic disorders include schizophrenia, schizo-affective disorder, bipolar disorder, drug induced psychosis. These disorders are characterized by wide spectrum of symptoms known as positive and negative symptoms. Positive symptoms such as hallucinations, delusions, disorganized speech, and agitated behavior are seen in psychotic disorders. In addition to positive symptoms, schizophrenia also features negative symptoms such as apathy, avolition and cognitive deficits. Schizophrenia is considered as a prototypic disorder for understanding the pathophysiology of psychosis and the impact of antipsychotic treatment. Antipsychotic drugs (previously known as'Neuroleptic agents') are used in

the treatment of these psychotic disorders. However the need in treating the various manifestations of psychotic illnesses is still unmet. The most important objective of designing animal model is to mimic the clinical condition of humans in the animals so that the therapeutic agents can be evaluated for their efficacy for the same clinical condition. Achieving this objective with construct, face, content and predictive validities in animal models, is very difficult for psychiatric illnesses. Usually the animal model will be chosen to mimic only some aspects among the broad symptom spectrum of the illness. Evaluation of antipsychotic agents is done by assessing the change in the normal behavior of the animals or drug induced behavioral changes in them.

Animal Models for Psychosis:-

❖ **Amphetamine Induced Stereotype in Rats:-[5]**

Amphetamine acts as an indirect sympathomimetic agent. It induces grooming; sniffing, licking and gnawing which exhibits stereotype behavior in rats. This can be prevented by using neuroleptics agents. This test predicts the efficiency and confirms the antipsychotic drug. Four groups (n=6) of adult Wistar rats were taken weighing 180 to 220 gms. They were treated with the standard drug (Haloperidol); treated concentrations 100,200,300 mg/kg of test extract respective groups and then placed in individual cages. They were administered i.p dose of d-amphetamine (5mg/kg) after 30 minutes. The onset of stereotypic behavior was observed at 30 minutes interval for 3 hours.

❖ **Phencyclidine (PCP) Induced Bizarre Pattern of Locomotor Activity:- [5]**

Phencyclidine is glutamate receptor antagonist. Locomotor hyperactivity gets induced in rodents by the subjection of phencyclidine and is alienated by antipsychotic drugs. Male Wistar rats were housed in a chamber. Animals were divided into groups (n=6), for test or the standard drug. 30 minutes prior the experiment, the animals were administered with the extract or the standard drug. Phencyclidine (2mg/kg) was administered to the animals of both the groups just before the start of the experiment. Photoactometer as shown in Figure was used to determine the locomotor activity for a period of 30 minutes. Drugs antagonizing the phencyclidine induced activity, act by some other receptor viz. glutanergic and serotonergic rather than dopaminergic receptors.

Figure 1:Monitoring of locomotor activity using Actophotometer [5]

❖ **PCP Induced Social Withdrawal Test: - [5]**

This test shows the efficiency of antipsychotic drugs against symptoms of schizophrenia. The time of social interaction in the rats is decreased by Phencyclidine. Before the start of the experiment, Wistar rats were kept in pairs for 10 days. During the test one, cage mate is removed and replace with new cage mate for 20 minute. The total amount of time spent on various elements of interaction i.e. social exploration, genital Investigation etc. is considered as the extent of social behavior. Phencyclidine was administered 5 minutes before the start of the experiment whereas the test or the standard drug was given 30 minutes.

❖ **Conditioned Avoidance Response in Rats:-[5]**

In the conditioned avoidance response paradigm, an electric foot shock is paired with a conditioned stimulus of a tone or light. This will elicit a learned avoidance response so the animal can escape being shocked. This link between the unconditioned and conditioned response is facilitated by dopamine, although several other neurotransmitters are thought to also play a role. When this dopaminergic pathway is disrupted, one sees a decrease in the normal, appropriate avoidance response (i.e., the animal fails to escape). This assay for a reduction in dopamine activity has been used to determine the efficacy of many dopamine antagonists, including both typical and atypical antipsychotics such as haloperidol and risperidone. Although this approach has been instructive in finding therapies that block dopamine

type 2 receptors and therefore are effective for psychosis, those very same agents actually impair a normal self-preservation function. Thus, the major limitation to this behavioral model is that its predictive validity is at odds with normal function, as well as lacking construct validity related to psychosis.

Figure 2:Monitoring of CAR using pole climbing apparatus [5]

❖ **Latent Inhibition:-[6]**

Latent inhibition (LI) refers to a process by which noncontingent presentation of a stimulus diminishes the ability to enter into subsequent associations. Thus, the prior experience that a stimulus does not have a consequence makes it less likely that the brain will form an association with that stimulus later. LI is widely considered to relate to the cognitive abnormalities that characterize schizophrenia because it reflects an organism's ability to ignore irrelevant stimuli. However, it also has been linked to psychosis based on the observation that medications that effectively treat psychosis, such as clozapine and haloperidol, restore latent inhibition across a variety of conditions.

❖ **Hyperactivity (behavioral measurement):- [6]**

Hyperactivity in animal models is a behavioral measurement that has been associated with the agitation and disorganized behavior of psychosis. Many early antipsychotics functioned dopamine agonists; therefore hyperactivity has been hypothesized to originate from a hyper-dopaminergic state. Hyperactivity, however, remains a consistent measurement even in models where dopamine release is not directly induced. It is suggested that the hyperactivity observed in such models is due to secondary effects on dopamine transmission. The maintenance of hyperactivity in models that do not directly influence dopamine supports the idea of elevated dopamine neurotransmission being characteristic of psychosis, but not necessarily the source of psychosis. Hyperactivity in mouse models was historically measured through first person observation. Presently, hyperactivity is measured through the use of computer software, yielding greater accuracy. In the former method, experimenters would conduct an open field test in which a grid was drawn on the floor and the experimenter would note every time a grid line was crossed by an animal, in order to calculate an ambulation score for locomotor activity. Locomotor activity can now be measured using a grid of photo-beams at the bottom of a chamber, which is combined with software that determines the number of beam breaks. Increased locomotor activity is often assumed to indicate a general level of hyperactivity.

❖ **Assessment of Novelty-Induced Rearing Behavior**:-**[7]**

Novelty-induced rearing behavior is used to assess the central excitatory locomotor behavior in rodents. Four groups of rat (n=7) were administered test extract (10 and 20 mg/kg, i.p.), chlorpromazine (1 mg/kg; i.p.), or isotonic NaCl (1 mL/kg, i.p.). One hour later, novelty-induced rearing behavior was explored by placing the animals directly from home cages to a transparent Plexiglas cage (45 cm × 25 cm × 25 cm). The rearing frequency (number of times the animal stood on its hind limbs or with its fore limbs against the walls of the observation box or free in the air) was recorded for 10 min. All rats were monitored individually by two observers who were blinded to the study groups. The area was cleaned with 5% alcohol to eliminate olfactory bias before beginning a fresh animal.

❖ **Apomorphine-Induced Stereotypic Behavior Test:-[7]**

Mesolimbic and nigrostriatal dopaminergic pathways play crucial roles in the mediation of locomotor activity and stereotyped behavior. Apomorphine-induced stereotypy is due to the stimulation of dopamine receptors and has been used a convenient method for in vivo screening of dopamine agonists or antagonists and assessment of dopaminergic activity. Briefly, four groups of rat (n=7) were administered minocycline (50 and 100 mg/kg, i.p.), chlorpromazine (1 mg/kg, i.p.), and isotonic saline (1 mL/kg, i.p.). One hour later, apomorphine (2 mg/kg, S.C.) was administered to each rat. First, rats were placed into the cylindrical metal cages (18 × 19 cm) containing vertical (1 cm apart) and horizontal (4.5 cm apart) metal bars (2 mm) with upper lid for 10 minutes for orientation period. After apomorphine administration, the rats were immediately placed back

into the metal cages and observed for stereotypic behavior. Signs of stereotypy, which include mainly sniffing and gnawing, were observed and scored as follows: absence of stereotypy (0), occasional sniffing (1), occasional sniffing with occasional gnawing (2), frequent gnawing (3), intense continuous gnawing (4), and intense gnawing and staying on the same spot (5). The stereotypic behavior was rated after each minute, and mean of 15 min period was calculated and recorded.

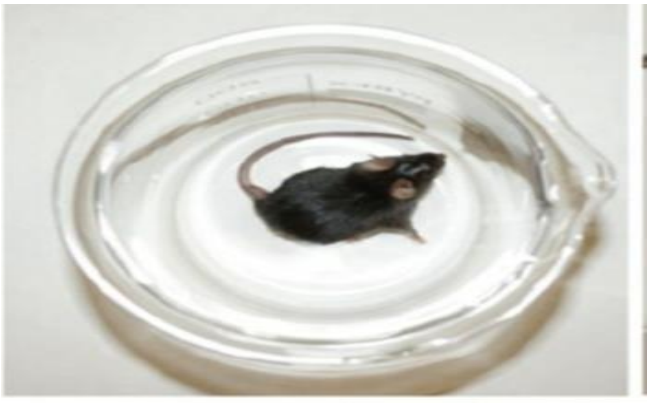

Figure 3: Apomorphine-induced rotation [8]

❖ **Novelty-Induced Stereotypy:- [9]**

The number and total duration of stereotypic behaviour exhibited by the mice pre-treated with test extract (10, 100 or 1000 mg/kg, p.o) or vehicle (distilled water, 10 ml/kg, p.o) over two-hour periods was assessed in an automated open-field test (VersaMax Animal Activity Monitoring System, AccuScan Instrument Inc., USA) as previously reported. This test system comprised four animal monitoring chambers (16 in × 16 in × 12 in) covered by transparent lids with perforations, an analyser and a computer. The base of each monitoring chamber was lined with vertical and horizontal laser generators and sensors. The behaviors of interest were pre-configured into. During the test, any behaviour exhibited by the test animals through beam interruptions were transmitted to the analyser, recorded on a computer, and the data subsequently exported to Microsoft Excel. Our experimental setup enabled the researcher to test the same animal under three different experimental conditions (primary, secondary and auxillary in succession). In the experiments, primary and secondary sessions were conducted for 60 and 120 min respectively. The test animals were made to acclimatize by undergoing a two-day procedure in the system without drug administration. On the third day, after an initial 60 min primary session, the mice were treated with test extractor distilled water (as a control) and tested for a 120 min secondary session. When the animal broke the same beam (or set of beams) repeatedly then the monitor considered the animal as exhibiting stereotypy. This typically happened during grooming, head bobbing, sniffing, gnawing, etc.

❖ **Apomorphine-Induced Locomotor and Rearing Activity:-[9]**

Using the experimental set-up as described in the novelty induced locomotor and rearing activities, mice were pretreated with test extract (100, 300 or 1000 mg/kg, p.o) or chlorpromazine (0.1, 0.3, or 1.0 mg/kg, i.p) or vehicle (distilled water, 10 ml/kg, p.o) and 30 min later, they received apomorphine (2 mg/kg, i.p) and placed in the open field test chamber. A vehicle group without apomorphine administration was also included. The events were recorded with a camcorder for 30 min and the videos tracked for the frequency of rearing and line-crossings.

❖ **Apomorphine-Induced Cage Climbing:-[9]**

This method as described previously was employed with minor modifications. Briefly, mice were treated with test extract (100, 300, 1000 mg/kg, p.o), haloperidol (0.1, 0.3, 1 mg/kg, i.p) or vehicle (distilled water, 10 ml/kg, p.o and injected with apomorphine (2 mg/kg, i.p) 30 min later and immediately placed individually into an all wire-meshed cage (mesh size: 1 cm × 1 cm; dimensions = 27 cm × 20 cm × 20 cm). A camcorder placed above the cage was used to record animal behaviour in the cage for 30 min post-apomorphine injection and the video recording tracked the frequency and duration of climbing. In a preliminary study done in our laboratory, haloperidol was found to be more potent than chlorpromazine in reducing the frequency and duration of cage climbing, thus in this assay haloperidol was used as the reference antipsychotic drug. In a separate experiment using the above experimental protocol, a combination of low dose test extract (100 mg/kg, p.o) and three doses of haloperidol (0.1, 0.3 and 1.0 mg/kg, i.p) were evaluated for synergism, addition or antagonism using the fixed ratio method.

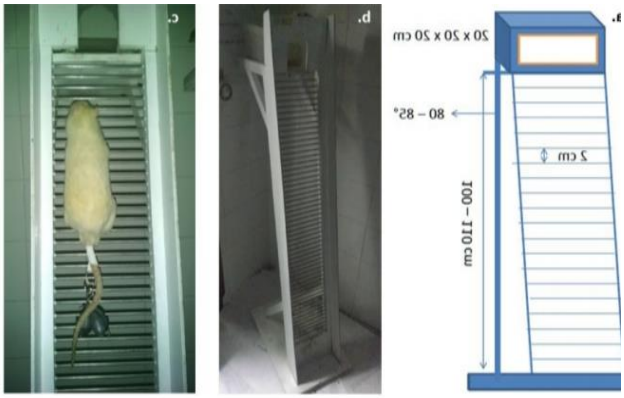

Figure 4: climbing equipment [10]

❖ **Ketamine-Induced Stereotypic Behaviour in Mice:- [11]**

In this model, animals were divided into seven groups, and each group consisted of six animals. The control group I received only saline (1 ml/kg, i. p) and negative control group II received ketamine (50 mg/kg, i. p). The animals of groups III, IV received haloperidol (1 mg/kg, i. p) and olanzapine (5 mg/kg, i. p) respectively and after 30 min ketamine (50 mg/kg, i. p) was given, for 21 consecutive days. Group V received test extract only (100 % v/v, p. o.) for 21 d. The animals of test groups VI and VII received different concentrations of test extract (50% v/v, 100 % v/v, p. o) respectively and after 30 min ketamine was given (50 mg/kg, i. p) for 21 consecutive days. Each mouse was individually placed into a separate plastic cage (37 × 24 × 30 cm), which was divided into quadrants by lines on the floor and was allowed to acclimatize for at least 30 min before the experiments. Behavioral tests were performed between 9 am to 4 pm. The stereotypic behaviour was assessed by counting the number of turning, weaving, and head bobbing counts. Turning was measured by counting turnaround attempt of each mouse every 10 min over 60 min periods. Weaving and Head-bobbing counts were measured by counting its neck movements towards the right and left, and up and down every 10 min over 60 min. Ataxia was assessed by counting the number of falls every 10 min over 60 min.

❖ **Pole Climbing Avoidance in Rat**:-**[11]**

In this model, animals were divided into five groups and each group consisted of six animals. The control group VIII received only the vehicle. The animals of groups IX and X received haloperidol (1 mg/kg, i. p) and olanzapine (5 mg/kg, i. p) respectively for 21 consecutive days. The animals of test groups XI and XII received different concentrations of test extract (50% v/v, 100 % v/v, p. o) respectively, for 21 consecutive days. The pole-climb avoidance paradigm is an avoidance-escape procedure used to separate neuroleptics from sedatives. Whereas sedative compounds suppress both avoidances and escape responding at approximately the same doses, neuroleptics drugs reduce avoidance at lower doses than those affecting escape responding. Male wistar rats weighing around 150 gm were used in the training and testing of the rat was conducted in the Pole climbing apparatus, which has a floor that acts as a source of shock. At the centre of the instrument, there is a wooden pole, which also serves as a shock free zone. The procedure and endpoint observed in the present study were as described earlier. Data were expressed in terms of the number of avoidance attempts and escape failures relative to the respective vehicle control group.

Figure 5: pole test [12]

❖ **Swim Induced Grooming in Mice:-[11]**

In this model, the control group XIII received only the vehicle. The animals of group IXV received haloperidol (1 mg/kg, i. p), for 21 consecutive days. The animals of test groups XV and XVI received different concentrations of test extract (50% v/v, 100 % v/v, p. o) respectively, for 21 consecutive days. Sixty minutes after treatments, mice were placed individually in swimming cylinders (8x8x18 cm high) filled with water (32 °C) for three min. They were then removed and dried with a towel for 30 seconds and placed immediately into Perspex boxes individually. The number and the total duration of grooming attempts were recorded over 15 min.

Figure 6: Swimming apparatus for mouse physical training [13]

Depression:-[14]

Depression typically presents as lowered mood, difficulty in thinking, loss of interest, and physical complaints such as headache, disturbed sleep, loss of energy, and change in sex drive. It incurs substantial personal, economic, and social costs for both the individuals afflicted and those close to them. Although synthetic drugs are easily available in the market such as tricyclic antidepressants.

Animal Models for Depression:-

➢ **Despair Swim Test:-[14]**

Mice were forced to swim, after 1 h of administration of test substances, in a Plexiglas cylinder (height 40 cm; diameter18 cm) containing water up to the level of 15 cm, and maintained at 25 ± 2 C (Kumar *et al.,* 2008). Mice were allowed to swim for 6 min. During this test period, the total duration of immobility (floating in the water in a slightly hunched but upright position, its nose above the surface) was noted.

Figure 7:Behavioural despair test [15]

➢ **Open Field Behavior Test:-[14]**

The apparatus was composed of a square wooden arena(40 cm 40 cm 40 cm) surrounded by a grey PVC wall. The floor was divided into 25 square marked by black lines. At the beginning of the test, the mice were 6 placed individually into the central part of the open field apparatus. The number of crossings and rearings was recorded for 5 min. The results have been expressed as mean ± standard deviation (SD). The test drugs were compared with standard drug and control by

one way analysis of variance (ANOVA) followed by Student–Newman–Keul's test.

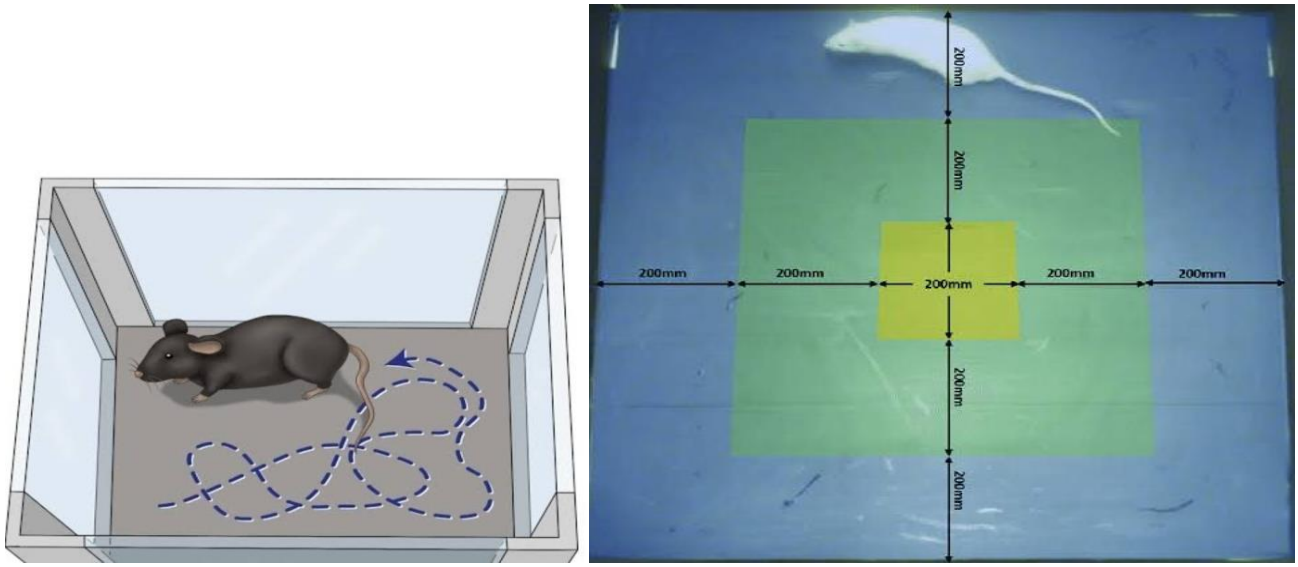

Figure 8:Open Field Locomotor Activity [16, 17]

- **Tail Suspension Test (TST):- [18]**

The TST was conducted according to the method of Steru *et al.,* Mice both acoustically and visually isolated were suspended by their tail in the TST apparatus (50 height × 45 width × 12 cm depths) on the first day and tenth day of drug administration. The duration of immobility was recorded for test period of 5 minutes. Mice were considered immobile when they hung passively and completely motionless.

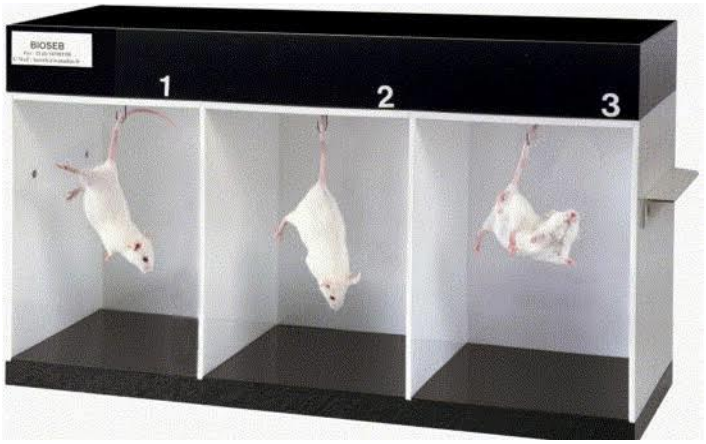

Figure 9: Tail Suspension Test [19]

- **Forced Swim Test (FST):- [18]**

According to the FST method described by Porsolt *et al.,* the mice were placed individually into 5 L glass beakers filled with 15 cm height of water. The water was changed frequently to eliminate fur, urine, and excrement after each test was done. When the mice remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above the water surface, they were considered to be immobile. This was classified as induced depression. The total duration of immobility was recorded during 5-minute test. The immobility period was calculated by subtracting total time (5 minutes) from time spent in escaping behaviour such as swimming and climbing. Swimming was defined as movements throughout the glass beaker and climbing was considered as upward directed movements of forepaws by the side of glass beaker. Antidepressant drug treatment reduced the length of time the animals remain immobile and increased the escaping behaviour.

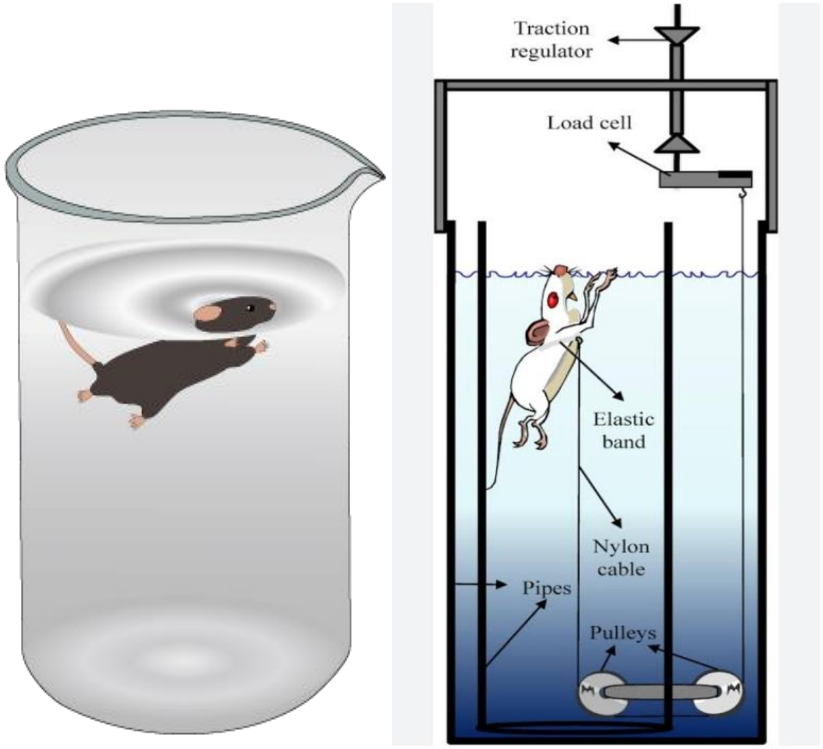

Figure 10:Forced Swim Test [20, 21]

➢ **Spontaneous Locomotor Activity (SLMA):- [22]**

The locomotor activity of animals was measured to distinguish between sedative and central nervous system stimulant activity of drugs. It was measured by using a digital photo actometer. After two doses of drugs 24, 5 and 1 hr before the test, mice were placed in the photo actometer covered with the lid made up of fiber. Mice tried to explore the area and during their movement they intercepted the photo beams. The number of interceptions was counted by the photoactive cells. Locomotion of the animal was expressed in terms of total number of ambulation (total photo beam counts) during a 5-minute test for each mouse.

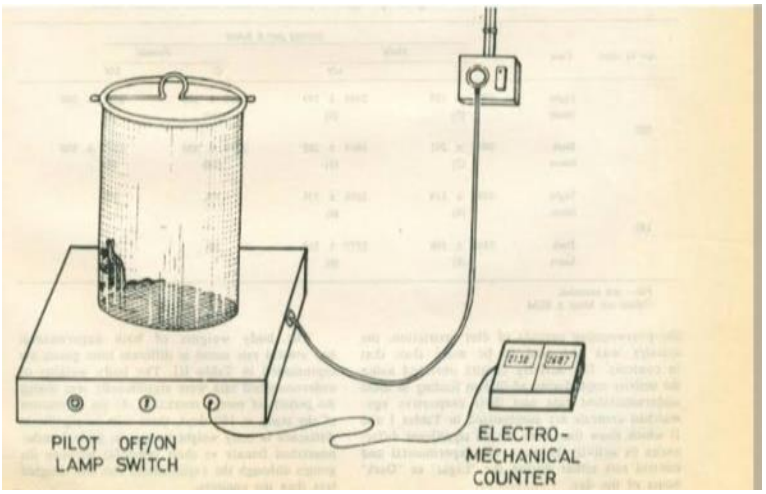

Figure 11:Spontaneous locomotor activity [23]

➢ **Shock Induced Depression:-[24]**

Mice were Shocked for 1 hour (0.75mA, 5sec duration at an interval of 10sec) on a metal grid floor. Activity was recorded in Actophotometer for a period of 10min before and after shock on 0 day and 8th day.

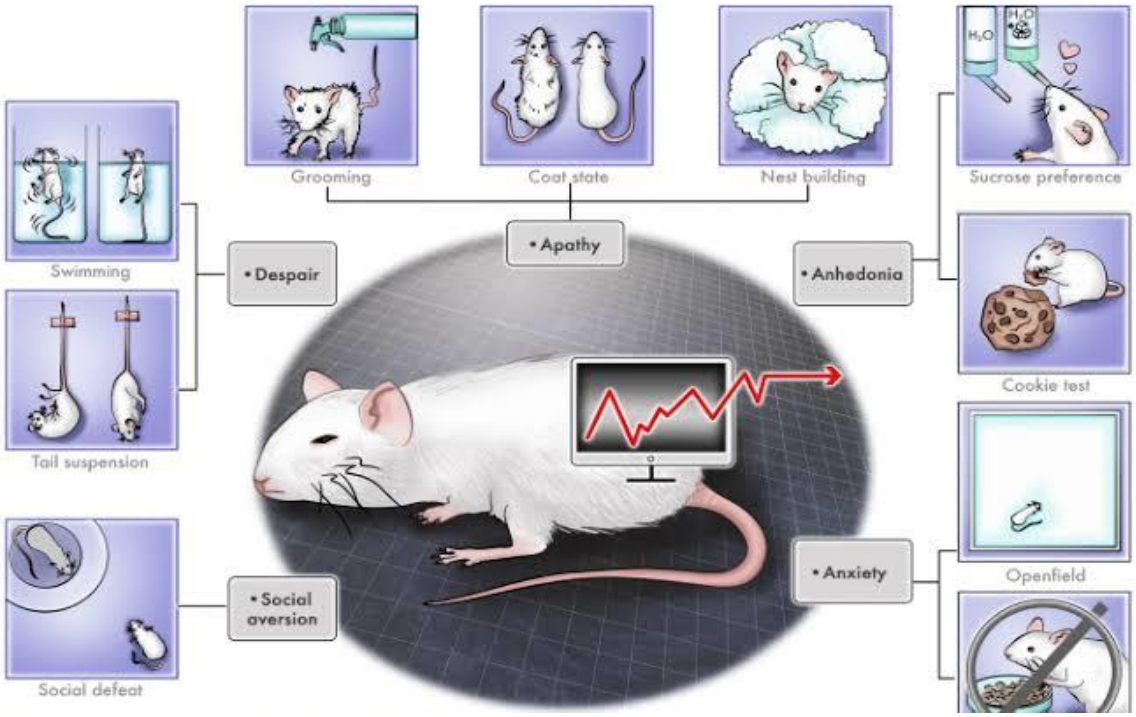

Figure 12: Behavioural endpoints measuring aspect of the anxio-depressive phenotype in rodents [25]

➢ **Chronic Mild Stress Model:-[26]**

Chronic mild stress is the most common cause of depressive mood disorders. It results in multiple physiological changes in the brain including the alteration of corticosterone regulation through the HPA (hypothalamus pituitary Adrenal) axis, impaired neurogenesis, synaptic dysfunction, and gene expression changes. It also induces behavioral changes including depressive-like behavior, a reduction in the reward response, and sleep disturbances. Several studies showed that animals exposed to repetitive stress display behavioral changes in open field behavior tests and a decrease in saccharin or sucrose fluid consumption, which is considered indicative of anhedonia. It was also shown that chronic, uncontrollable stress contributes to the impairment of the brain stimulation reward system. Therefore, these reports suggest that the chronic stress animal model can be used to study depressive neuropathology and several studies used this model for the test of therapeutic targets to treat the depressive disorder. In the mouse model of chronic mild stress, the mice are subjected to unpredictable mild psychosocial stressors for 9 weeks marked expression changes in brain region including the prefrontal cortex and hippocampus, in chronic mild stress animal models Terms related to mitochondria and membranes were enriched in the increased genes group, for the prefrontal cortex, but the same terms were identified in the decreased genes group, for the hippocampus. Related to this result, many studies have shown the connection between mitochondria and depression. Moreover, the GO (gene ontology) terms related to neurogenesis were enriched in the increased genes group, for the hippocampus. Neurogenesis may be altered in animals with chronic stress.

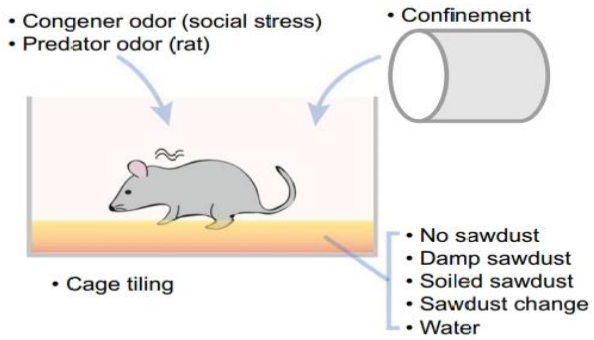

Figure 13:Chronic mild stress-In this model, the mice are exposed to a series of low-intensity stressors at unpredictable times for 9 weeks [26]

➢ **Chronic Social Defeat Model:-[26]**

The chronic social defeat animal model has been used to study the pathology of depression and its underlying mechanisms. The chronic social defeat model is characterized by decreases in locomotor activity, reductions in enthusiastic and aggressive behavior and increases in submissive behavior and anxiety, as is observed in humans with depression. These symptoms ultimately lead to an increased risk of depression progression. Furthermore, morphologically, the chronic social defeat model featured a reduction in neuronal cell proliferation and a decrease in hippocampus volume. It was also demonstrated that the chronic social defeat model could alter reward circuit and cause changes in the brain, associated with increased susceptibility to engaging in depressive behavior. Moreover, the chronic social defeat model altered the activity of dopaminergic neurons in the ventral segmental area and ultimately resulted in social avoidance and a reduced preference for sucrose, as is expected in depression pathology. Other studies also showed that chronic social defeat stress leads to functional and structural changes in neural circuitry. In particular, it was demonstrated that the ventral hippocampus and nucleus accumbens were more susceptible to stress from chronic social defeat than was the prefrontal cortex. In the previous work, it was established that chronic social defeat stress induces susceptible and resilient phenotypes in a ratio of 2 to 1, respectively. The susceptible phenotype showed enduring social avoidance, and there silient phenotype exhibited a tendency of social interaction similar to control mice. Therefore, the expression data of susceptible phenotype were only used for the following analysis. Based on the transcriptome of the prefrontal cortex, hippocampus, nucleus accumbens in the chronic social defeat model, the result of GO (gene ontology) analysis for each gene group is presented. Moreover, the mitochondrion term was also detected in the decreased genes group for the hippocampus. These results are quite similar to those observed in the chronic mild stress model. However, the terms related to neurogenesis and the myelin sheath was enriched in the decreased genes group for the prefrontal cortex in the chronic social defeat model. Some of these terms were identified in the increased genes group for the hippocampus in the chronic mild stress model. Further, the small molecule metabolic process term was the most highly enriched in the increased genes group for the hippocampus in the chronic social defeat model. The same term was the most highly enriched in the decreased genes group for the prefrontal cortex in the chronic mild stress model. We suggest that chronic mild stress and chronic social defeat models have both common and opposite molecular alterations in the prefrontal cortex and hippocampus, respectively. For the nucleus accumbens, in the chronic social defeat model, the terms related to RNA and ribosomes were included in the increased genes group while those related to neurogenesis and membranes were included in the decreased genes group. Because the neurogenesis was decreased both in the prefrontal cortex and thenucleus accumbens in this model, it is reasonable to expect that a similar molecular change, related to decreased neurogenesis, occurs in these areas.

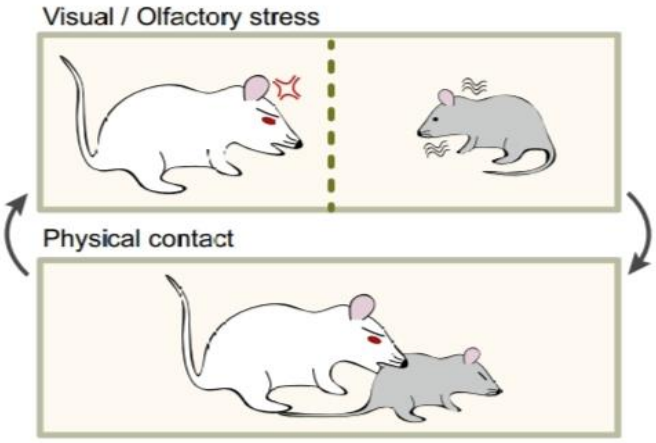

Figure 14: Chronic social defeat- In this model, depression is induced over 10 days by directly exposing the experimental mouse to a larger and aggressive mouse for 5 minutes a day and then housing across a transparent barrier to sustain sensory contact [26]

- **Physical Pain Model:-[26]**

Physical pain is another major cause of depression. Neuropathic and nociceptive pain, especially, increase the risk of developing depression .Approximately one-fifth of the general population currently suffers from chronic pain. Specifically, pain caused by damage to sensory nerve pathways has been shown to influence depressive moods and to be involved in neuronal cell death at brain regions linked to depression, including the insular lobe, prefrontal cortex, thalamus, hippocampus, anterior cingulated, and amygdala. It was reported that the prefrontal cortex and nucleus accumbens experience neuronal cell death during pain, which subsequently led to the development of depression. The nucleus accumbens is connected to several brain regions related to depressive-like behavior

and pain regulation, including the ventral segmental area, thalamus, prefrontal cortex, and amygdala. Several neuroimaging studies have demonstrated that patients suffering from chronic physical pain differed from healthy individuals in terms of activity in the nucleus accumbens and prefrontal cortex, which play a role in reward processes. Synaptic dysfunction was also reported in the prefrontal cortex, due to neuropathic pain, in the relevant animal model. It has also been found that chronic physical pain is strongly associated with areas ranging from the ventromedial prefrontal cortex to the periaqueductal gray. This region represents the control center for descending sensory pain modulation and has pain reducing enkephalin-producing cells in humans and rodents. Thus, chronic pain and depression show common changes in neuroplasticity mechanisms and are strongly linked to each other. The physical pain model can provide more understandable information toward developing treatments for depression. The GO(gene ontology) analysis using the transcriptome of the prefrontal cortex and nucleus accumbens in the chronic pain mouse model 2.5 months after the injury was performed. Interestingly, the GO (gene ontology)term "catalytic complex" was enriched both in the increased and decreased genes groups for the prefrontal cortex and nucleus accumbens. However, there was no notable overlap of GO (gene ontology)terms between the physical pain model and other models. This maybe is because the physical pain model is a model of depression induced by physical surgery, whereas psychological stimulation is induced in the other depression models.

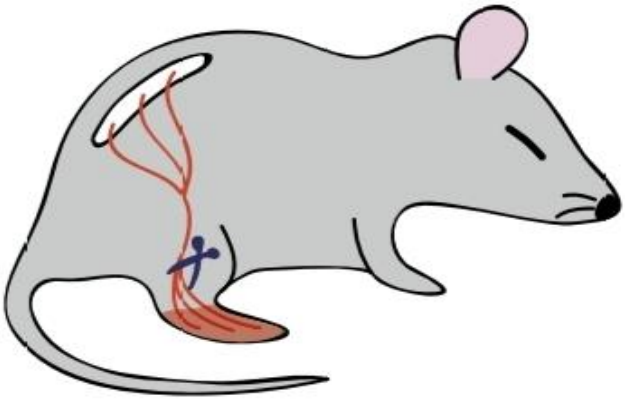

Figure 15:Physical pain (spared nerve injury)-A spared nerve injury is surgically inflicted, resulting in depressive behaviors due to persistent neuropathic pain. [26]

- **Learned Helplessness Model:-[26]**

The learned helplessness model has been used to make predictions in cases of depression because it accounts for the symptoms of traumatic stress disorder and comorbid major depression. Learned helplessness features symptoms of depression that affect neurochemical and molecular processes. These include increased inflammation and the cell death of nor epinephrine neurons in the locus coeruleus region, leading to depressive behavioral consequences. In the learned helplessness mice model, 360 scrambled electric foot shocks (0.15 mA) with varying duration (1–3 seconds) and interval (1–15 seconds) are treated for two consecutive days. From the GO analysis of the transcriptome from the learned helplessness model, the most enriched terms in the decreased genes group, for the hippocampus, included those related to the synapse). A previous study reported on the remodeling of synapses in the learned helplessness depression model. However, no GO (gene ontology) terms that were enriched in the learned helplessness model were identified in the GO(gene ontology)analysis of the hippocampus in other depression models. We expect that this depression model is characterized by different molecular changes in the hippocampus, compared with other depression models including the chronic mild stress and chronic social defeat models. Based on the description above, it is obvious that there are commonalities and differences across the many animal models of depression, in terms of changes in gene expression profiles and depressive pathology, in each brain region. One of the notable conclusions from the GO(gene ontology) analysis is that the difference in gene expression among depression models is greater than that among tissues that we analyzed such as the prefrontal cortex, hippocampus, and nucleus accumbens Thus, although there were many functional terms commonly affected across the different models, our findings indicate that we should consider the difference among the depression animal models, and that it is important to choose a proper model to study depressive disorder. We found that chronic mild stress and chronic social defeat models show very similar molecular changes for the gene group with the greatest changes in gene expression. In contrast, the physical pain model had no specific terms in common with the other depression models. This suggests that the selection of a

suitable model is required based on the type of depressive disorder that the researcher wants to study.

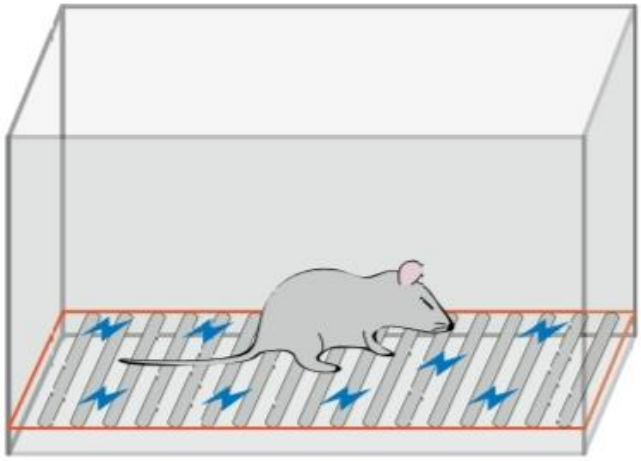

Figure 16: Learned helplessness-The mouse is exposed to unpredictable and inescapable electric footshocks for two consecutive days, after which the mouse shows a defect in its escape behavior and depressive symptoms [26]

Anxiety:-[27]

Anxiety disorders are one of the most prevalent and highly comorbid psychiatric conditions. They are among the most common mental, emotional, and behavioral problem. Anxiety is a normal, emotional, reasonable and expected response to real or potential danger.

Animal Models for Anxiety:-

- **Spontaneous Motor Activity:-[28]**

The animals were divided into 14 groups, with each group constituting 6 mice. For spontaneous motor activity, every mice was introduced into the Actophotometer (INCO) and its score of locomotor was measured for 10 min duration.

Figure 17: spontaneous motor activity wheels [29]

- **Elevated Plus-Maze Model:-[27]**

EPM test for studying the anxiolytic effect in rodent was used. EPM consists of two open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm × 40 cm) with an open roof. After oral administration of drugs, the rat was placed in the center of the maze, facing open arm for 1 h of elevation at 50 cm. During a 5 min test period the following measures were taken: average time spent by the animal in the open arms (average time = total time spent in open arms/number of entries in open arms); and total number of entries in open arms. During the entire experiment, the animals were allowed to socialize. Every time before placing the animal, the arena was cleaned with 5 % alcohol to eliminate the possible bias due to the odor left by the previous animal. Every precaution was taken to ensure that no external stimuli could invoke anxiety in the animals.

Figure 18:Elevated plus-maze test apparatus [30]

➢ **Light-Dark Model:- [27]**

Two compartment exploratory models of Crawley and Goodwin has been validated pharmacologically, behaviorally and physiologically. The two compartment method titrates the natural tendency of rat to explore a novel environment, against the aversive properties of brightly light open field. The time spent in light area seems to be the most reliable parameter for assessing anxiolytic activity. The light and dark box consists of two compartments: one light area (27L × 27× 27H cm) was illuminate by 100 W desk lamp was painted white and the other dark area (18L × 27W × 27H cm) was painted black. The two compartments were separated by partition with tunnel (7.5 × 7.5 cm) to allow passage from one compartment to other. Thirty min after the administration of the test drug, each animal was individually placed in the center of the light compartment (facing away from the door). During 5-min test period, number of transitions, and time spent in light zone were noted.

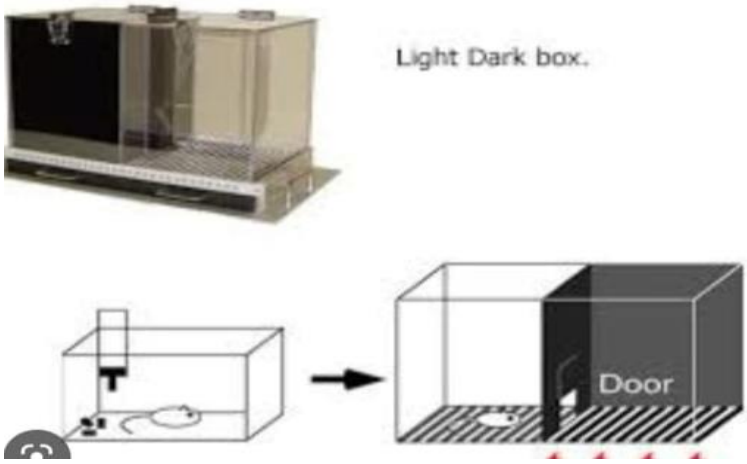

Figure 19: Light and dark box [31]

- **Open- Field Test (OFT):- [32]**

Albino mice (20-30 g) of either sex were divided into 05 groups of 06 mice in each was fasted overnight prior to the test but water was supplied ad libitum. Group I was maintained as normal control received vehicle only once daily for 7 days, group II was received diazepam (1 mg/kg, p.o.), and Groups III, IV and V were treated with different doses of test extracts p.o. respectively once daily for 7 days. On 7th day 60 min after administration of the vehicle, standard and test extract, and each mouse was placed in the center of open field arena and the following parameters were recorded during a test session of 5 min. Ambulation: Measured in terms of the number of squares crossed by the animal and Rearing: Number of times, the animal stood on its hind limbs.

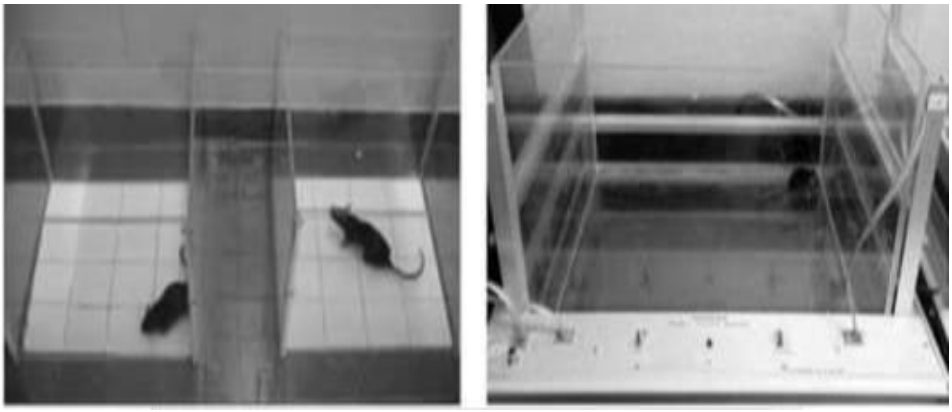

Figure 20: Open- Field test [33]

- **Social Interaction Test:- [34]**

Swiss albino mice weighing 20-30 grams are divided into four groups of six mice each. The standard drug (Diazepam) was given 30 minutes before testing, and the extracts were given 45 minutes before testing. They were housed alone for 5 days before the trial and were given unlimited food and water. During this time, they were weighed and handled regularly. The location of the cages in the rack was adjusted to ensure that all rats had equivalent exposure to the various levels of illumination. The mice were allocated to the „low light and unfamiliar' test settings at random. The text box was (22 *15 * 12 cm) in size, and pairs of mice were placed in it for 5 minutes, and their behaviour was monitored. Sniffing, nibbling, grooming, following, mounting, kicking, boxing, wrestling, and leaping on, under, or over the companion were all observed.

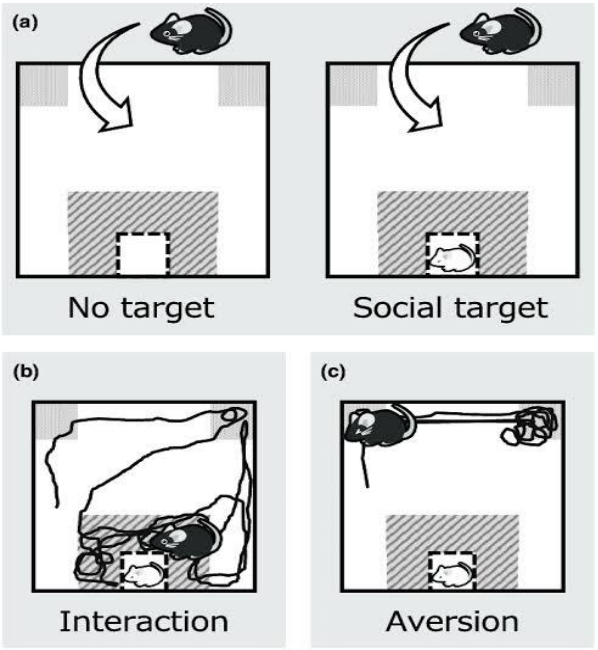

Figure 21: Social Interaction test [35]

- **Hole Board Test:- [34]**

The hole board test is designed to assess particular head dipping habits. The hole board equipment consisted of an open field with 16 evenly placed 3 cm diameter holes in the floor. Swiss Albino mice were split into four groups of six mice each. The standard medication diazepam was given i.p.30 minutes before testing, and the extracts were given p.o. 45 min before testing. Each mouse was placed in a different corner of the board, with the animal then wandering about and dipping its head into the holes. Individual mice had their head dips and sectional crossings counted every 5 min.

Figure 22: Hole Board test [36]

- **Elevated T maze**:- **[37]**

The elevated T maze (ETM) was originally proposed by Graeff *et al.,* It is based on the EPM and consists of three arms: one enclosed by a lateral wall standing perpendicular to two opposite open arms of equal dimension. The whole apparatus is elevated from the floor. This model allows measurement of two different behaviors in the same animal: the conditioned response represented by inhibitory avoidance of the open arms and the unconditioned response represented by escape behavior when the animal is placed in the extremity of these arms. These responses have been related to generalized anxiety and panic disorders, respectively. The ETM was developed in response to the inconsistencies found in other animal models of anxiety, particularly the EPM, regarding drugs that interfere directly with serotonergic neurotransmission. On the day before the test, animals are exposed to one of the open arms of the T-maze for 30 min. This prior forced exposure to one of the open arms of the maze decreases the latency to leave this arm on a later trial. This result has been attributed to the habituation of behavioral reactions to novelty, which may interfere with one-way escape. Twenty-four hours after pre-exposure to the open arm; the animals are tested in the ETM to measure inhibitory avoidance acquisition. To this end, each animal is placed at the distal end of the enclosed arm of the ETM facing the intersection of the arms. The time taken by the rat to leave this arm with all four paws is recorded (baseline latency). The same measurement is repeated in two subsequent trials (avoidance 1 and 2) at 30-s intervals. Following avoidance training (30 s), each rat is placed at the end of the same previously experienced open arm and the latency to leave this arm with all four paws is recorded for three consecutive trials (escape 1, 2 and 3) with 30-s intertribal intervals. A cut-off time of 300 s is usually established for the avoidance and escape latencies.

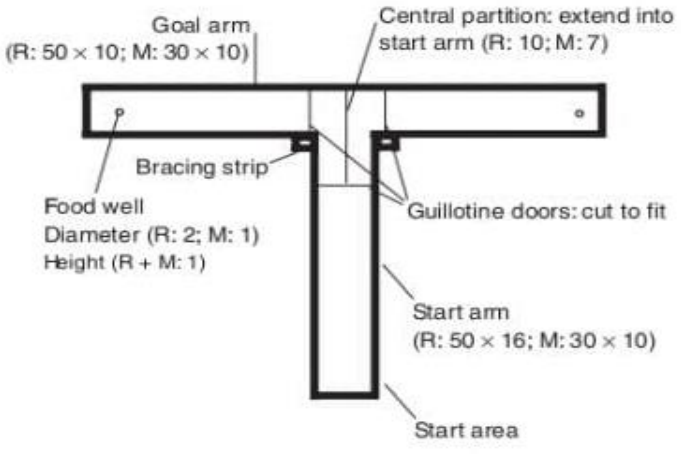

Figure 23:T-maze plan. Dimensions are in cm: R = rat, M = mouse, for enclosed mazes, walls should be 20 cm high (mouse), 30+ cm (rat); for elevated mazes, 1 cm (mouse), 3 cm (rat) [38]

- **Vogel Water-Lick Conflict Test**:- **[39]**

This test is a well-known method used in rats, designed by Vogel *et al.,* Only few studies tried to apply the test to other species, but recently, this test has been reported to successfully detect anxiolytic-like action of diazepam and to be appropriate as a screening method for drugs that have apparent anti-anxiety actions. In this test, thirsty animals gain water reward through a water spout, but at the expense of receiving a mild electric shock delivered to the tongue. Licking in controls is suppressed, anxiolytic release this suppressed behaviour, while non-specific effects are assessed on non- punished water drinking. Diazepam and pentobarbital produced a significant anti-conflict effect, which means that these drugs increased the number of electric shocks mice received during the test session. Yohimbine, caffeine, scopolamine, cyclazocine cimetidine, baclofen, MK-801, buspirone, chlorpromazine and haloperidol all did not produce anticonflict effects in this test using ICR mice. L838, 417, a novel GABA was anxiolytic in this mouse model, using C57BL/6. Nevertheless, it seems difficult to set up control experiments for locomotor activity, nociception, learning, memory, etc. Different strains of mice have been validated with this task.

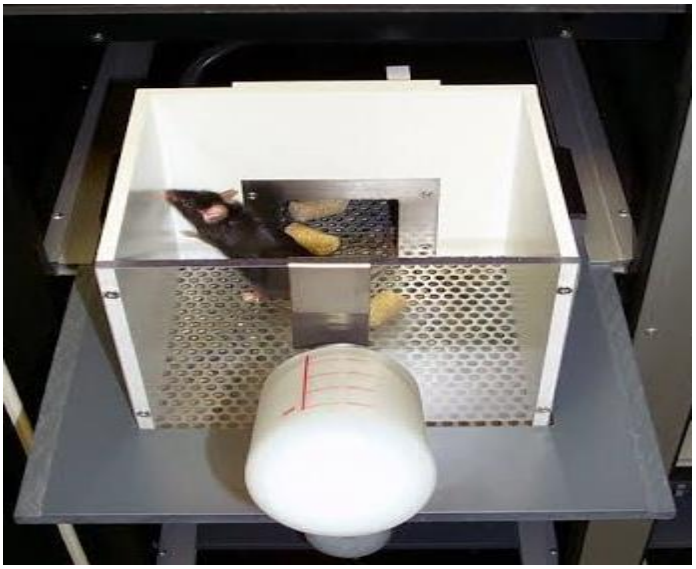

Figure 24: Vogel test [40]

- **Four-Plate Test**: - **[39]**

The four-plate test (FPT) introduced by Boissier *et al.,* is based on the suppression of a simple innate ongoing behaviour, i.e. the exploration of novel surroundings, of the mouse. The apparatus consists of a floor made of four identical rectangular metal plates. This exploration behaviour is suppressed by the delivery of mild electric foot shock contingent on quadrant crossings. Every time the mouse crosses from one plate to another, the experimenter electrifies the whole floor evoking a clear flight-reaction of the animal. BDZs increase the number of punished crossings accepted by the animal. 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. This is verified utilizing a hot-plate apparatus, employing morphine as the control compound. This paradigm is not commonly used in behavioral studies, making it difficult to formulate inter-laboratory comparisons. As such, the various factors potentially influencing the behavioral response of mice have not been profoundly studied. However, its success in our laboratory and the demonstration of an anxiolytic-like effect of ADs in this model (in comparison to many of the traditional paradigms employed) emphasizes the validity of this model. In addition, the FPT allows the exploration of anxiety underlying mechanism such as an inter-regulation between 5-HT2-subtype receptors and a2 noradrenergic receptors. Our laboratory reported that a single prior undrugged exposure to the FPT reduces punished responding on retest at intervals ranging from 24 h to 42 days. Furthermore, prior experience attenuates the anxiolytic response to the BDZs diazepam and lorazepam, similar to results observed in the EPM and L/D. However, the FPT is now being increasingly used for the detection of the anti-anxiety activity of potentially new anxiolytic.

Figure 25: the four plate test in mice [41]

- **Fear-Potentiated Startle**: - **[39]**

Originally designed by Brown *et al.,* in 1951, this pavlovian fear conditioning procedure involves two different steps. First, the animals are trained to associate a neutral stimulus, generally a light, with an aversive stimulus such as an electric foot-shock. After training, animals are submitted to an intense sound. The startle response to this unconditioned stimulus is potentiated by simultaneous presentation of the previously conditioned light stimulus. This potentiation can be found even 1 month after the training. Anxiolytics produce a dose-dependent reduction of the startle amplitude with no change in the baseline level of the startle (observed in the absence of the conditioned stimulus). A decrease in the baseline would reveal a non-specific locomotor impairment. Main results of this model have been published by Davis *et al.,* Overall, BDZs, as well as buspirone-like drugs, decrease fear-potentiated startle, often without any change in the baseline response. Administration of some antidepressants (imipramine, fluvoxamine or amitriptyline), acutely or chronically, seems to exert no effect in this model. This model has little predictive value for anxiogenic treatments, as yohimbine and FG-7142 reduced startle response and these data corroborate the recent hypothesis that systems mediating fear-potentiated startle are independent from systems mediating increased startle from unconditioned and putatively anxiogenic stimuli.

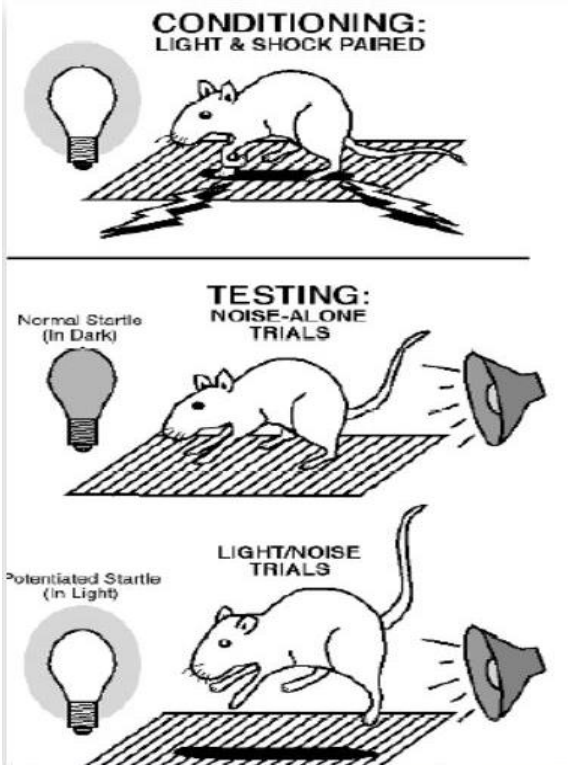

Figure 26:fear potentiated startle [42]

- **Marble Burying Test**:- **[43]**

Marble burying (MB) behavior was assessed based on published methods. The testing apparatus consisted of a plexiglass mouse cage (internal dimensions: 33 cm long 21 cm wide 19 cm high) filled to a depth of 5 cm with corncob bedding (Rahi Agro, Industries, Ahmedabad, India) and placed in a sound-attenuating room lighted by white light. Prior to each test, 10 clear, glass marbles (10 mm diameter) were evenly spaced and arranged in a grid-like fashion across the surface of the bedding. Then, each mouse was placed into the observation cage. At the conclusion of the 60 min test, the mice were carefully removed from the chamber and the number of buried marbles (50% or more of the marble was covered by bedding) was determined.

Figure 27:Marble Burying Test [44]

- **Defensive (Shock-Prod)Burying Test**:- **[45]**

Similar to the marble burying test, this paradigm is another pharmacologically sensitive method to assess rodent anxiety. Mice usually bury noxious stimuli posing an immediate threat (e.g., electrified shock-prod). The test has pharmacological validity, as benzodiazepines and the serotonergic anxiolytic potently suppress shock-prod burying in a dose-dependent manner, where as anxiogenic drugs have been proven to increase this behavior.

1. In a standard-sized mouse cage with bedding5 cm deep, insert a wire-wrapped prod (6–7 cm long) through a hole 2 cm above the bedding surface.
2. After the initial contact with the bare wires and the subsequent shock, record the behavior of the animal for10–15 min. Behavioral measures of activity may include prod-directed burying, burying latency, height of pile at prod base, prod contacts (number, duration), prod contact latency, and stretch-attend postures directed at the prod.

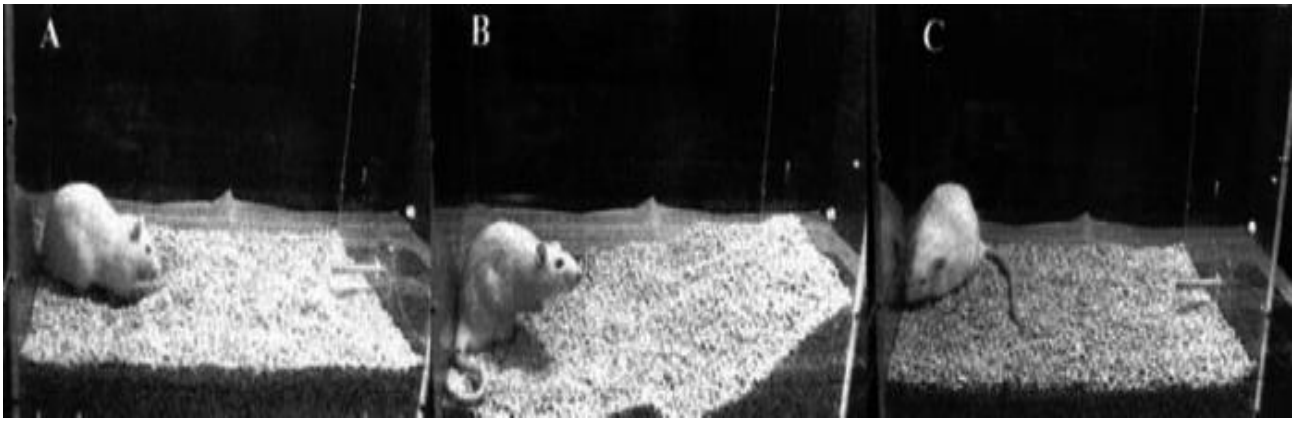

Figure 28: Photographs depicting animals in the defensive burying test. (A) Some time after receiving a shock, animal begins spraying bedding toward the prod. (B) Typical of the F344, animal engages in extensive burying behavior such that bedding completely covers prod. (C) Unlike the F344, the WKY buries very little and after a series of prod approaches, remains immobile in a corner of the test chamber [46]

- **Rat Exposure Test:- [45]**

This test utilizes the natural defensive "avoidance" behavioral response of mice to signs of potential danger, such as a natural predator (e.g., rat). Defensive behaviors include stretch attend posture, stretch approach, freezing, burying, and hiding and are measured as a function of risk assessment. This test has proven useful to determine strain differences in defensive behaviors and relative levels of anxiety in response to predators. Additionally, the defensive behaviors measured are sensitive to anxiolytic, making this paradigm useful in pharmacological screening.

1. Introduce the mouse into the small black box, which will serve as a "home chamber" (safe environment). The Plexiglas tunnel should allow free movement between the home chamber and the observation box.
2. On the first 3 days of testing, allow the mouse to explore the observation box for 10 min to become familiar with the environment. In these sessions, there should be no rat present.
3. On the fourth day, insert the mouse into the home chamber and the rat into the observation box for 10 min. The rat should be placed in the opposite side of the cage isolated from the mouse by the wire mesh.

Figure 29: Photograph of rat exposure test apparatus. The predator (rat) is placed in the right half of the exposure cage, which is divided into two equally sized compartments by a wire mesh screen (surface and predator compartments) [47]

Mania:-[48]

Mania—Lasting at least a week and causing significant impairment in social and occupational functioning or requiring hospitalization. Bipolar disorder is a serious, chronic mental illness characterized by unusual changes in mood, energy, and activity levels. Early diagnosis and appropriate treatment of bipolar disorder are important because the illness carries a high risk of suicide and can severely impair academic and work performance, social and family relationships, and quality of life.

Animal Models for Mania: -

- ✓ **Amphetamine Induced Hyper locomotion (AIH):- [49]**

The AIH test is a widely used test for the detection of antimanic activity. We administered amphetamine and waited 0, 20, 40 and 60min before subjecting the mice to the locomotor activity measurement procedure. Thus, 24h after the second habituation session, mice were treated with saline or amphetamine 3.0mg/kg(both i.p.) and tested in the activity chamber immediately following20min, 40min or 60min. Beam interruptions were measured over a period of 20min.we administered drugs in combination with amphetamine and compared the locomotor activity to control groups. Twenty-four hours after the second habituation session, mice were treated with either vehicle (S.C.) or an experimental drug (lithium, tamoxifen or medroxyprogesterone, all S.C.) and 15min later were administered with either saline or amphetamine 3.0mg/kg (both i.p.).Twenty minutes after the amphetamine administration, the mice were tested in the activity chamber for 20min. Intracerebroventricular (i.c.v.) administration of chelerythrine was performed under ether anaesthesia 20min before amphetamine administration. In brief, a 0.4-mm external diameter hypodermic needle attached to a cannula, which was linked to a 10-µl Hamilton syringe, was inserted perpendicularly through the skull and no more than 2mm into the brain of the mouse. A volume of 0.2µl was administered over a period of 25s and the needle remained in place for an additional 25s to avoid a reflux of the injected substances. The injection site was 1mm to the right or left of the mid-point on a line drawn through to the anterior base of the ears. To ascertain that the drugs were administered exactly into the cerebral ventricle, the brains were dissected and examined macroscopically after the test. Animals with bleeding or error in the injection site were discarded from analysis (two animals were discarded).

✓ **Pre-Pulse Inhibition (PPI):- [50]**

Four sound-attenuated boxes (SR-Lab Startle Response System, San Diego Inst., San Diego, USA) were used. Each box consists of a Plexiglas cylinder situated on the top of a platform with a sensor that detects the strength made by the rat in each trial. Two speakers situated 15 cm from each side of the cylinder deliver the acoustic stimuli and a white noise generator provides the background noise. Each box is constantly lit by a 10 W lamp. The data are transduced by an accelerometer into a voltage which is amplified, digitized, and saved into a computer for further analysis.

The startle session starts with a 5min habituation period in the startle chambers. Then, 10 "pulse-alone" trials (105 dB, 40ms) are delivered in order to obtain a basal measure of the ASR (BASELINE 1). After this, each one of the six different types of trials are randomly administered 10 times (60 trials in total):

(1) Pulse-alone trials (105 dB 40ms, BASELINE 2, this was the variable used to calculate the %PPI; see the formula below).
(2) Prepulses of 65/70/75/80 dB (20ms) followed by the startle stimulus (105 dB, 40ms), with an inter-stimulus interval of 100ms.
(3) No stimulus trials (background noise 55 dB). At the end, in order to measure the habituation to the startle stimulus, five "pulse-alone" trials were delivered (BASELINE 3). The interval between trials was 10–20 s with a mean of 15 s. The startle magnitude was recorded during 200ms after the onset of the pulse. The degree of PPI (in percentage) is calculated according to the formula:

$$\%PPI = 100 - (\textit{startle amplitude on prepulse trials/ startle amplitude on pulse trials} \times 100)$$

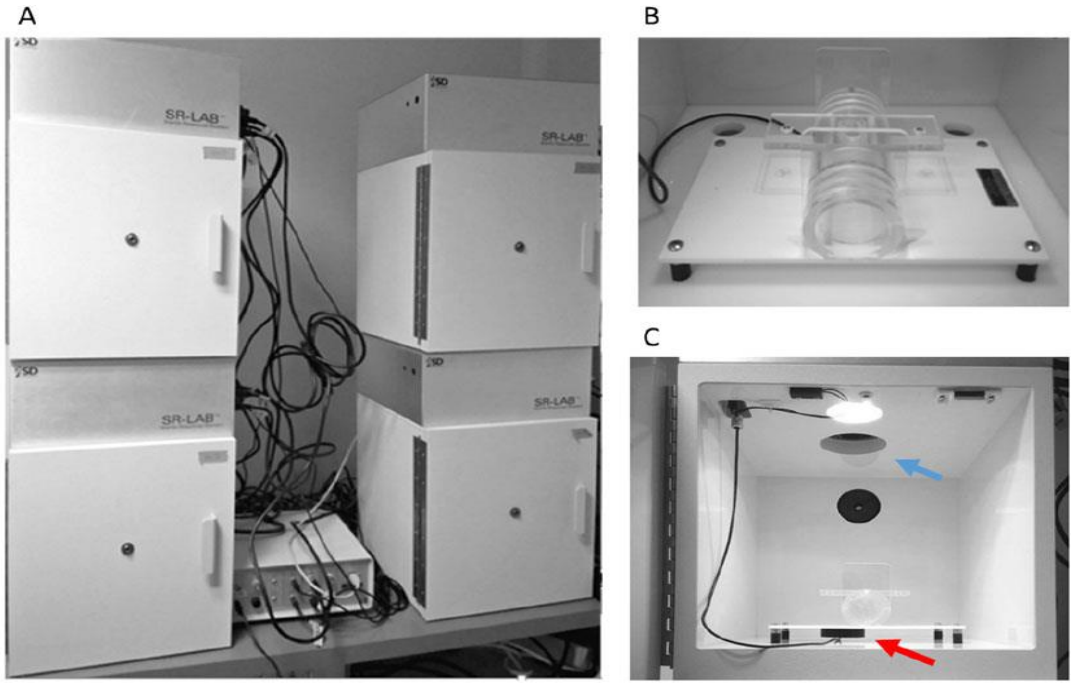

Figure-30:- SR-LAB startle apparatus

(A). Each experimental apparatus consists of an outer, lighted and ventilated, chamber that serves to prevent external noise or vibrations interfering with experiment.
(B). Inside the chamber a stabilimeter consisting of a Plexiglas cylinder is secured to a platform.
(C). A piezoelectric accelerometer-indicated by the red arrow-mounted under the cylinder transduces animal movements that are then digitized, rectified, and recorded by a computer and interface assembly. A loudspeaker-indicated by the blue arrow-generates the startling acoustic stimuli, according to the desired settings [50]

✓ **Open Field Test:- [51]**

The open-field area was made of acrylic (with transparent walls and a black floor, 30 cm x 30 cm x 15 cm) divided into nine squares of equal areas (Archer, 1973). The apparatus was placed in a red light room. The animals were gently placed on the left corner and allowed to freely explore the arena for 5 min. The number of squares crossed was used to determine locomotor activity (crossings), while rearing behavior was used as parameter of exploratory activity. Two experienced raters who were blind to the treatment regimen analyzed the behavior of each animal.

Figure 31: open field activity [52]

✓ **Predator Odor Test**:- **[51]**

This test took place under red light illumination in a clear Plexiglas testing arena measuring 40 cm long x 27 cm wide x 36 cm high. The floor of the arena was wire mesh, and no lid was used during testing. An alligator clip was securely mounted 3 cm above the floor in the center of one of the front walls of the arena. A hide barrier measuring 24 cm (long) x 24 cm (wide) x 22 cm (high) was placed against the opposite end wall (Deisenberg and McGregor, 2001). A small opening in the front of the hide barrier provided entry into the barrier to as a protective environment to mice. The animals were first exposed to a habituation trial (13th day), with duration of 10-min. At the beginning of habituation trial, each mouse was individually placed into the arena with its nose in close proximity (2–4 cm) to the alligator clip. During the trials, no collar material was attached to the clip. On the testing day (14th day), cat odor was obtained by rubbing a damp cloth (18×22 cm) vigorously against the fur of a domestic female adult cat for 5-min. This procedure was carried out 1 h prior to the experimental session. The cat odor cloth was kept in a sealed plastic bag. Each cloth was used for one exposure only. Damp pieces from the original cloth, not rubbed on the cat, were used for the neutral odor. All odor exposures took place in a separate, small, dimly lit room, and neutral odor exposures always preceded the cat odor exposures in order to prevent any traces of cat odor influencing the neutral odor group. The testing arena and hide barrier were washed with unscented laboratory soap and water after each mouse. For each animal, collar testing was performed during 5 min. The parameters recorded were: the number of cat cloth contacts, the time of cat cloth contacts and the duration of sheltering events. Cloth contact was defined as direct contact or sniffing ≤ 5 cm from the cloth. The sheltering event was defined when the animal entry with the four legs in the protective area behind the hide barrier.

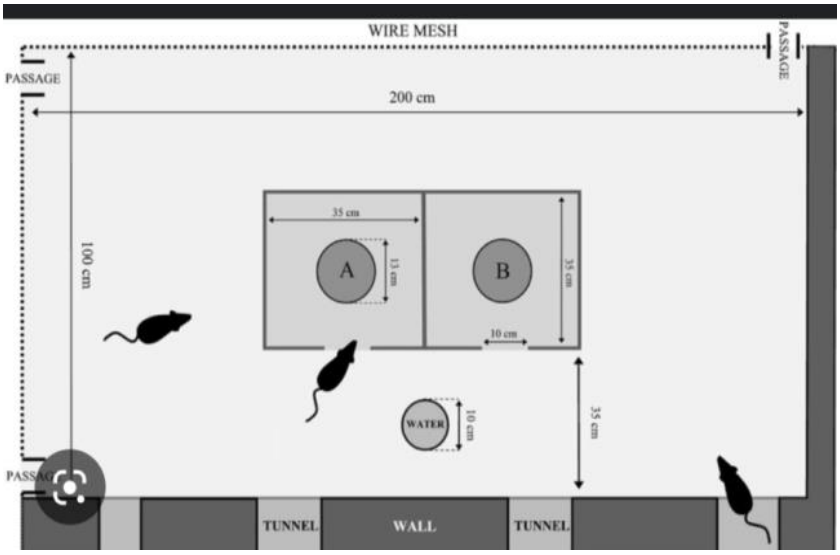

Figure 32: Experimental arena in which the wild rat colony was observed. (A, B) Flat, rubber bowls, where 15 pellets of laboratory feed were provided at the same time of day on a daily basis. During experimental sessions, cardboard pieces at the bottom of bowl A or bowl B were soaked with predator urine [53]

✓ **Spontaneous Alternation in the Y-Maze:-[54]**

Each mouse was placed at the end of one arm of the maze and allowed to freely move through the apparatus during 8 min. The apparatus consisted of a Y-shape maze with three white, opaque plastic arms (length 35, width 5, and wall height 10) at a 1201 angle from each other arms. A correct alternation was defined as entries in all three arms on consecutive occasions. The percentage of correct alternations was calculated as follows: total of alternations/ (total arm entries2).

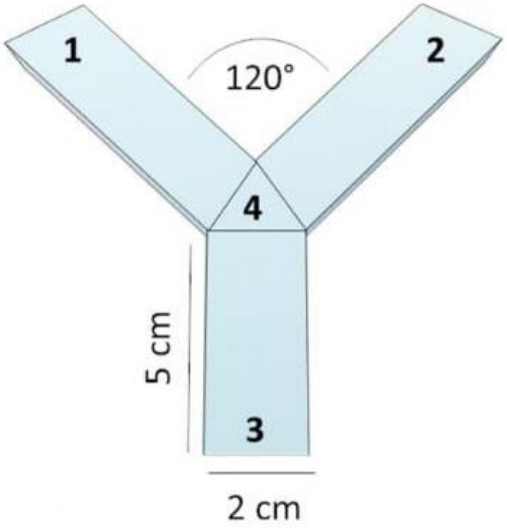

Figure 33:Y-maze [55]

✓ **Sweet Solution Preference Test:- [56]**

Mice were supplied with a bottle of 1% saccharin solution (SIGMA, St. Lewis, MI, USA) on top of the regular supply of water and food. Saccharin concentration was selected as it is in the high end of the concentration-intake curve and based on previous work done with BS (Black Swiss) mice. The saccharin solution bottle was available to the mice throughout the entire sweet solution preference test period. Weights of saccharin solution and water bottles were taken at the beginning of the experiment and every 24 hours. Sweet solution preference was calculated daily as the ratio of saccharin out of total liquid consumption. For Stage 1, saccharin preference was measured across 4 days and for Stage 2 it was measured across 2 days because it was demonstrated that differences between groups were established within the first 2 days.

Figure 34: Sucrose preference test [57]

✓ **Resident-Intruder Test**:- **[56]**

Resident mice were transferred in their home cages to an experimental room where cage covers were removed. After a 5-min adaptation period, a younger, previously group-housed mouse (i.e., intruder), was placed into the resident's cage and behavior was digitally recorded from above for a 10-min session. Recordings served to manually score resident's aggressive interactions (defined as attempts to bite, actual bite, boxing postures and wrestling postures) and non-aggressive interactions (defined as other types of body contact including sniffing, all grooming and body contact). Behaviors performed when not interacting were not scored. The resident's aggression score was calculated as the ratio of aggressive interactions out of total (aggressive + non-aggressive) interactions. At the end of the session the intruder was removed and placed back in its home cage, the resident's cage was covered and both mice were returned to the colony room. To minimize harm to animals, mice were briefly separated when attacks became vicious and included significant biting. Hence, the total time of attacks could not be scored and only numbers of aggressive and non-aggressive interactions were scored from recordings. This method was previously shown to be sensitive enough to demonstrate the effects of mood stabilizers in this test.

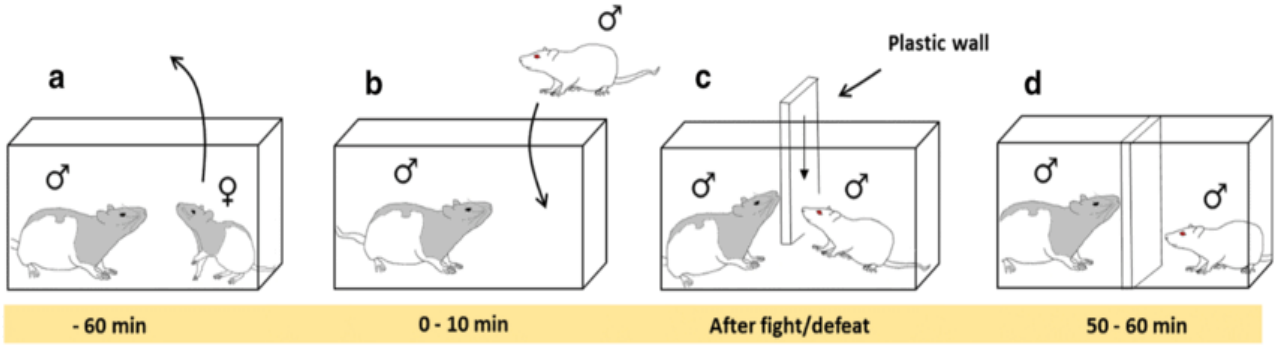

Figure 35: Resident-intruder paradigm.

The set-up used to induce defeat stress in the intruder Sprague–Dawley rats (white) in the cage of the resident male Long Evans rat (black-hooded).

a) The Long Evans female was removed 60 min prior to the experiment.
b) An intruder Sprague–Dawley rat was placed in the home cage of a resident male Long Evans rat.
c) Upon three episodes of social defeat (submissive supine posture, freeze or fight), or after 10 min of interaction, a plexiglas wall was used to separate the resident and intruder rats.
d) Sensory interaction in the divided cage was allowed for the remaining time of the hour. Both the male Sprague–Dawley and female Long Evans rats were returned to their home cage after the conditioning [58]

✓ **Amphetamine-Induced Hyperactivity:- [56]**

Mice were administered with amphetamine or vehicle and immediately placed in activity monitors for a 60-min session, where activity was detected by infrared beams and total ambulatory activity was calculated across the entire session. At the end of the session mice were returned to their home cages and the boxes were wiped clean with a 10% alcohol solution.

✓ **Preventative Treatment:- [59]**

In the prevention model, we simulated the maintenance phase of BD (bipolar disorder) treatment, according to a previously proposed protocol. In brief, different groups of animals were treated with Li-lithium (47.5 mg⁄kg i.p.), ALA-Alpha-lipoic acid [50 or 100 mg⁄kg orally (p.o.)], or saline once a day for 14 days. Between the eighth and the 14th days, Li-, ALA-, and saline-treated animals additionally received on daily injection of either AMPH-Amphetamine-2 mg⁄kg or saline i.p. The time interval between dugs administration was 30 min. Locomotor activity was measured two hours after the last injection (14th day).

✓ **Reversal Treatment:- [59]**

In the reversal model, we reproduced the treatment of an acute manic episode according to previously proposed protocol. In brief, the animals received one daily injection of AMPH- Amphetamine-2 mg⁄kg or saline i.p. for 14 days. On the eighth day of treatment, the animals in the saline and AMPH groups were divided into four groups, receiving one daily injection of the following drugs, with a 30-min interval between treatments: Group (i) AMPH 2 mg⁄kg in saline 0.9%; Group (ii) AMPH 2 mg⁄kg plus ALA 50 mg⁄kg, i.p.; Group (iii)AMPH 2 mg⁄kg plus ALA 100 mg⁄kg, i.p.; Group (iv) AMPH 2 mg⁄kg plus Li 47.5 mg⁄kg, i.p. On the 14th day of treatment, the locomotor activity was assessed two hours after the last injection.

✓ **Amphetamine Sensitization**: - **[60]**

The animals were pre-treated with either d-amphetamine (1.8 mg/kg) or vehicle for 5 consecutive days. After an 17–19 day withdrawal period the animals were treated with drug or vehicle and placed individually in makrolon locomotor activity cages (20

cm × 35 cm × 18 cm) to habituate for 30 min. After the habituation period, a low dose d-amphetamine challenge (0.9 mg/kg) or vehicle was administered and locomotor activity was recorded for 30 min. The locomotor activity cages were equipped with 5 × 8 infrared light sources plus photocells. The light beams crossed the cage 1.8 cm above the bottom of the cage. During the test session, locomotor activity was recorded as crossings of infrared light beams, and total locomotor activity was the accumulated number of crossings over the 30-min period. The recording of a motility count required interruption of two adjacent light beams, thus avoiding counts induced by stationary movements of the mice. All experiments were conducted during the light phase of the cycle, and initiated using a clean cage.

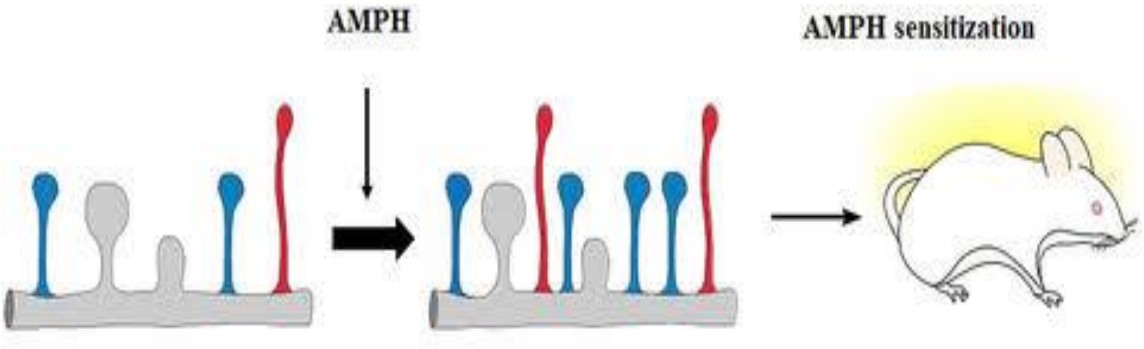

Figure 36: Amphetamine sensitization [61]

✓ **Morris Water Maze Test:- [62]**

The Morris water maze test was a spatial learning and memory test. The mice were trained 5 consecutive days daily with four trials, during this period, the mice were trained from different a starting quadrant to locate and escape onto the platform. The platform position was fixed throughout the test. Animals that failed to find the location within 60 seconds were guided to the platform and were allowed to remain on it for 20 seconds. On the sixth day, the platform was removed, and the mice were given 60 seconds to explore, and the time spent in the target quadrant was collected for each mouse.

Figure 37: Morris water maze test [63]

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