Name of Unit	Introducton to Dosage calculations and Screening of CNS Models
Subject /Course name	Experimental Pharmacology
Subject/Course ID	BP810ET
Class: B.Pharm. Semester	VIII
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Learning Outcome of Module 02

LO	Learning Outcome	Course Outcome Code
LO 1	To remember and understand the basic principles of preclinical screening models, including dose selection, dose calculation, and preparation of drug solutions or suspensions.	BP810.2
LO 2	To understand and explain the importance of grouping of animals and the role of sham, negative, and positive control groups in experiments	BP810.2
LO 3	03 To apply the rationale for selection of appropriate animal species and sex for pharmacological studies	BP810.2
LO 4	To analyze and evaluate different preclinical screening models used for drugs such as, nootropics, anti-Parkinson's, and CNS active drugs.	BP810.2

Content Table

Topic
• Dose selection, calculation and conversions, preparation of drug solution/suspensions, grouping of animals and importance of sham negative and positive control groups. • Rationale for selection of animal species and sex for the study. • Study of Screening Animal Models For : • Nootropics • Anti-Parkinson's • CNS activity- analgesic, antipyretic, anti-inflammatory • General anaesthetics • Sedative and hypnotics • Antipsychotic • Antidepressant • Antiepileptic • Alzheimer's disease.

DOSE SELECTION, CALCULATION, CONVERSIONS AND PREPARATION OF DRUG SOLUTIONS/ SUSPENSIONS

INTRODUCTION

Dosage selection, calculation and stock solution/suspension preparation based on dosage rationale formula are prerequisites to drug administration in experimental animals. As the literature is silent on the exact procedure for drug dosage calculations and stock solution preparations to guide the administration in experimental animals, it becomes challenging for the undergraduate, post-graduate students and other researchers during designing the protocol. Considering the most commonly employed animals i.e. rats or mice for conducting the in vivo experiments in medical, physiological, pharmacological, chemical, toxicological, biological, biochemical and genetic studies, the present chapter describes the calculation of doses, preparation of stock solution for experimental animal.

1. **Vehicle, drugs dissolution and volume:**

Vehicle:

A vehicle can be defined as a substance that acts as a medium in which a drug is dissolved and administered. Thus, to ensure that only the drug should induce the effect in all animal research, it is mandatory that a vehicle should be biologically inert. Moreover, it should also be per se non-toxic to the animals. The most commonly choice of vehicles for animal research are distilled water, normal saline (0.9% sodium chloride), 50% polyethylene glycol, 5 to 10% Tween 80, 0.25% methylcellulose or carboxymethylcellulose (Karl-Heinz et al., 2001; Nebendahl, 2000).

Dissolution and volume

i. Oral route calculations:

Generally, in pre-clinical studies involving experimental animals, a stock solution of the test drugs dissolved in appropriate volume of solvent (vehicle) is prepared and from it the dosages are calculated. Depending on the choice of vehicle, OECD (organization of economic corporation and development) has recommended some guidelines for dose calculation. As per these guidelines:

(a) With non-aqueous solvent/vehicle in oral route of administration, the dosage of drug (mg) should be constituted in an appropriate volume not usually exceeding 10 ml/kg (1 ml/100 g) body weight of

experimental animals (mice and rats).

(b) With aqueous solvents/vehicle, 20 ml/kg (2 ml/100g) body weight can be considered.

Example for maximum volume calculation:

As per 10 ml/kg volume selection: For a 100 g rat the required dose volume can be calculated as follows: animal wt (g)/kg × volume of stock (ml)

1 kg = 1000 g

Thus, 1 ml of volume from the drug stock solution maximum should be administered to a rat of 100g. Similar calculation can be performed for any volume selection (ml/kg) as per the body weight of the animals (OECD, 2000).

High dose volume problems:

However, if the dose volume is exceeded for administration more than recommended (40 ml/kg body weight), then animal might undergo stress. Moreover, the extra volume might overload the stomach capacity and pass immediately into the small bowel or can result in passive reflux in the stomach, aspiration pneumonia, pharyngeal, esophageal, and gastric irritation or injury with stricture formation, esophageal and gastric rupture and stress

Dosage calculation and preparation of stock solution for experimental animals:

Thus, considering the volume selection of 10 ml/kg, if a researcher wants to administered a dose 200 mg/kg to rat with body weight 100g the stock solutions and doses of drug can be calculated as follows.

Dosage calculation:

Body weight of rat = 100 g

Formula Calculation for 200 mg/kg dose/100 g rat

Design in mg = Body weight of animal (g)/1000*DOSE(MG)

Dosage in mg = 1OOg/1000*200mg =20mg

Thus 20 mg of test drug is required to be administered to 100g of rat to get a dose of 200 mg/kg. Therefore, a 20 mg of the drug needs to be dissolved/constituted in an appropriate vehicle for administration.

DISSOLUTION OF DOSE IN SUITABLE VEHICLE FOR ORAL ADMINISTRATION

As per the OECD guidelines, 100 g rat requires 20 mg of the drug, which should be constituted in not more than 10 ml of normal saline (see calculation for 10 mL/kg volume) according to the OECD guideline. That means, for 100 g of rat, 20 mg drug to be dissolved in 10 ml of normal saline. As in case of animal experimentation, large numbers of animals are needed to be administered with the same dose/different dose. Therefore, it is advisable to prepare a stock solution of drug from which all animals can be treated. See the following section for preparation of stock solution.

PREPARATION OF STOCK SOLUTION

Bulk volume of the stock solution required can be calculated by multiplying both sides by a constant value as follows:

20 mg = 1.0 ml

40 × 20 mg = 40 × 1.0 ml

800 mg = 40 ml

800 mg of drug will be dissolved in 40 ml of normal saline.

i.e.

800 mg/40 ml = 20 mg/ml

Thus, the final concentration of the stock solution that should be prepared is 20 mg/ml and this will be required for selected dose of 200 mg/kg for a rat of 100 g. Therefore, a volume of 1 ml can be administered from the stock solution and that will contain 20 mg of the drug required for a rat of 100 g to achieve a dose of 200 mg/kg.

Similarly, a stock solution of the drug for a higher selected dose of 400 mg/kg can also be prepared by dissolving 800 mg of drug in half of the volume (20 ml). Thus, stock solution with a higher concentration (40 mg/ml) will be produced. In this case animals with similar body weight from two different selected dose categories (200 mg/kg and 400 mg/kg respectively) will receive the same volume, but of different concentrations.

CALCULATIONS FOR INTRAPERITONEAL OR SYSTEMIC ADMINISTRATION

Dosage calculation (example: caffeine):

Caffeine is a CNS stimulant at a standard dose of 30 mg/kg for rat via intraperitoneal route as reported in several experimental literatures (Jain et al. 2005). The required dose of caffeine to induce CNS stimulation intraperitoneally in a rat weighing 200 g at a standard dose of 30 mg/kg can be calculated as follows:

Required dose for 200 g rat = Weight of animal (g)/1000 g × Standard dose (mg)

i.e. 200 g/1000 g × 30 mg = 6 mg

Therefore, 6 mg of caffeine is required to be administered to a 200 g rat to achieve a dose of 30 mg/kg.

DISSOLUTION OF DOSE IN A SUITABLE VEHICLE AND PREPARATION OF STOCK SOLUTION FOR INTRAPERITONEAL ADMINISTRATION

Dissolution:

As per the guidelines, an appropriate volume of vehicle ranging between 2 ml/kg to 5 ml/kg in rats is recommended for parenteral route like intraperitoneal administration.

Based on 5 ml/kg volume selection, in this example, 6 mg of caffeine would be constituted in 200 g/1000 g × 2 ml = 0.4 ml of a vehicle (normal saline) corresponding with the volume required for 200 g rat.

Thus, considering the OECD's guidelines for volume of 2 ml/kg, 200 g rat requires 6 mg of the drug, which should be constituted in not more than 0.4 ml of normal saline. That means, for 200 g of rat, 6 mg of drug is to be dissolved in 0.4 ml of normal saline. As in case of animal experimentation, large numbers of animals are needed to be administered with the same dose/different dose. Therefore, it is advised to prepare a stock solution of drug from which all animals can be treated. See the following section for preparation of stock solution.

Preparation of stock solution

To prepare a stock solution for more experimental animals, both the drug weight (6 mg) and the volume (0.4 ml) are multiplied with a constant value as per the number of animals or administrations required. For example if 10 animals or administrations are planned then both sides are multiplied with 10:

Stock solution concentration: 15 mg/ml.

Thus, for 10 numbers of experimental animals the quantity of caffeine required is 60 mg and it is constituted in 4 ml of vehicle to yield a bulk stock solution of 60 mg/4 ml with final stock solution concentration of 15 mg/ml. Therefore, a volume of 0.4 ml can be administered from the stock solution and that will contain 6 mg of the drug required for a rat of 200 g to achieve a dose of 30 mg/kg i.p.

Note: This approach would be able to conserve and estimate the quantity of drug required for a given population of animals. However, dissolution volume of 5 ml/kg can be considered for intraperitoneal administration in mice (due to their low body weight compared to rats).

POINTS TO BE REMEMBERED BEFORE APPLYING THE ABOVE APPROACH FOR DOSE CALCULATION AND STOCK SOLUTION PREPARATION FOR ANY ROUTE OF ADMINISTRATION

- The solubility of the drug needs to be checked from an appropriate monograph in the chosen vehicle.
- While calculating the dose of a drug with salt form, base calculations are done for that drug to ensure the administration of exact amount of pure drug from the salt form (e.g. in 1 g of caffeine hydrochloride what amount pure caffeine is present – molecular weight of salt/molecular weight of free drug).
- Precise weighing of drug is recommended using high precision digital weighing balance for stock solution preparation.
- During dispensing the required volume from the stock solution for drug administration using syringe, exact volume should be taken with due care to remove the air bubble completely from the syringe before adjusting the final volume of administration.
- The researcher should be well trained in the procedure of drug administration

RATIONALE FOR SELECTION OF ANIMAL SPECIES AND SEX FOR THE STUDY

INTRODUCTION

Scientists who are planning experiments evaluate both animal and non-animal approaches. The appropriate species is selected based on various scientific and practical factors, including the following:

Scientific Factors

Before choosing an animal species for a particular research project following scientific aspects are generally considered:

- The selection of species is generally based on the critical review of the scientific literature revealing, which species have provided the best, most applicable historical data with type of the research project under consideration.
- The species that will yield the most scientifically accurate and interpretable results.
- The species is also chosen on the basis of that the proposed experiments will be most relevant and useful to present or future investigators in perform on it.
- Moreover, a species which have special biologic or behavioral characteristics that make them most suitable for the planned studies.
- The species have features that render them inappropriate for the planned studies.
- The species that present the fewest or least severe biologic hazards to the research team.
- The species that require the fewest number of animals to complete the study.

Apart from the above general aspect of animal species selection some more important scientific factors that need to be considered before choosing an animal species are

1. Appropriateness as an analogue:

Researcher needs to ensure that the part or organ being studied has a function similar to the target species in applying research-derived data

2. Transferability of Information:

Using a specific animal species/model is to define a process in a system with the hope of transferring the data gained to a more complex system. Traditionally, one-to-one modeling is sought: modeling in one group of species/organisms that can be transferred to another group/species that has several analogous features of interest. This is especially helpful in modeling disease states.

3. Generalizability of results:

The choice of the species to be used in an experimental study also greatly depends on the ability of it to generalize results to the target species that is human. It is well known that this species is genetically highly variable, with cultural, dietary, and environmental differences. Thus, the importance in pharmacological

and toxicological modeling and has opened up the new field of research in pharmacogenetics. Therefore, genetic various among the species should be considered for a particular type of activity to be screened, while selecting a animal species.

4. Ethical Implications:

The most important aspect of choosing an animal species for an experimental study is to get a ethical clearance by justifying using an animal in it. It is generally recommended by the federal guidelines i.e CPCSEA that the use of alternate methods other than the animal use is should be considered if alternative methods are available. Alternate methods could consist of using cell lines, bacteria, computer models, or even human volunteers (Russell, 1959).

5. Numbers needed:

For the post-experimental data analysis, the number of animals required for a study is important. Thus, a biostatistical analyst should be consulted prior to submitting a proposal. Therefore, depending upon the numbers of animals needed the choice of animal species will vary. As numbers provide scientific validity especially for publication, it will impact many other factors such as cost and housing availability.

6. Customary practice within a particular discipline:

Choice of an animal also depends on the accurate genetic, microbiological, physiological, or psychological facets associated a animal and which are customary for a type of study under consideration. Thus, customary practices for a type of study generally justify and support the other criteria listed for a satisfactory and faster route of choosing the animal species for an investigation.

Animal Care Factors

1. Cost and availability:

Choosing an animal for a study is certainly depend upon the cost and availability factor. However, the choice of an animal should not be completely done on the base of cost but the above listed scientific factors should also be considered. Cost also includes ongoing care not only for husbandry but also for experimental manipulation.

2. Housing:

Before choosing an animal species, the available animal housing facility at the place of research is also cardinal. Example: choosing mice for a study is more feasible as it can housed in several

number in cages/room as compared to a nonhuman primate that might require new caging and hiring of additional personnel to provide specialized husbandry care.

3. Stress factors:

It is the most important factor that can affect the outcome of any study if ignored. As stress is known to affect the animal's physiology, biochemistry, and behavior. Sources of stress can be transportation, handling and manipulations, overcrowding, lack of environmental enrichment, and the research project itself. Therefore, the animal species, which are more stable for type stress that it might encounter in a planned study should be chosen.

Animal-Related Factors

1. Genetic aspects:

Generally, it is observed that some animal species are inbred in some laboratories and leads to inbred genotype that is known as genetic drift. Therefore, while choosing animal species for a study the uniformity of organisms is necessary, where applicable. Otherwise, it can affect the conclusions from the comparable research results obtained in different laboratories due to the use of own subline of the same inbred strain .

2. Background knowledge of biological properties:

The researcher should be completely aware with the biological properties of the animal species such as generalized and specialized function of body components, because it aids in the decision of whether the chosen animal is a spontaneous model or it needs to must be experimentally induced. For example, a rat would not be the best choice in biliary studies due to the absence of a gallbladder.

3. Ease of and adaptability to experimental manipulation:

In some case researcher chooses an animal species for performing a experimental method that is impossible on that species. For instance guinea pigs have highly inaccessible blood vessels and would be impractical in studies requiring repeated blood sampling. Therefore, choice of animal species also depends on its ability to adapt for the experimental manipulation required during a study.

4. Size of the animal:

The size of the animal employed also play a cardinal role in the choice of animal. Size is not only important with respect to its impacts on housing and husbandry availability but also considered for the requirement of tissue sampling or blood collection, physiological or morphological properties such as joint strain or organ size during a study.

5. Life span and age:

The impact of life span or age of animal on studies requiring components certainly affect the species chosen. Thus, studies with lesser time the animal species with smaller lifespan like rats & mice can be chosen. On the other hand, for long or chronic studies animal with longer life should be chosen eg. chows monkeys 20-30 years.

6. Sex:

The alternating cycle of hormonal production during oestrous cycle in the female gender of animals have been reported to affect the outcome of many studies. Therefore, male animal have been the choice of researcher for obtaining the steady results in 90 % cases. However, any study should be performed using both the sex considering the applicability of it in both sex of humans. However, certain studies are exclusively performed on female due to absence of the system or target physiology in males e.g. study related to pregnancy, teratogenicity, contraception, lactation, breast cancer etc. Thus, while planning for the research project the influence of sex difference on the outcome of the study on the data outcome must be considered.

7. Progeny Needed

Sometimes the study is designed on the progeny of the animal species. Therefore, the number of progeny produced by an animal species also is consideration for the choice of the animal.

Overall, all the above factors should be considered for making a righteous choice of the best animal species for the proposed research project. It is recommended that before choosing a animal species for a study researcher should consult with other scientists who have already used the animal species. Moreover, consultation with those responsible for housing and maintaining the animals will be helpful for a proper care of the animal and its physical environmental needs and animal-related factors.

ANTIANXIETY MODELS

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

According to DSM 5 anxiety disorder is classified into 3 major types.

- *Anxiety disorders which consist of separation anxiety, selective mutism, specific phobia, social phobia, panic disorder, agoraphobia, and generalized anxiety disorder*
- *obsessive-compulsive and related disorder which consist of body dysmorphic disorder, hoarding disorder, excoriation disorder,*
- *trauma and stressor related disorder which consist of post-traumatic stress disorder, acute stress disorder and adjustment disorder.*

Fear is not the only symptom in anxiety disorder as seen by the above classification. This classification is based on the underlying relationship between the disorders and their similarity in symptoms and the co-occurrence among them. Neurobehavioral animals model have been vital in understanding the pathogenesis of a disorder and discovering novel pharmacological strategies to treat them. But still these models are under argument that animals do not behave in same as humans and various behaviors which are considered similar to symptomatology of a disorder is not always accepted by the researchers at all times. Still animal models of emotional disorders try to obtain three types of validity; face validity is the ability to reproduce features of psychiatric disorder, the ability to detect clinically effective pharmacological agents shows the predictive validity, and the similarity in the etiology of the disease in humans and animals gives the construct validity.2 Anxiety is a complex disorder with different etiology and involves different neurochemical systems. Animal model for specific for each anxiety disorder are difficult to develop, hence various models can be used to screen a compound efficacy, and to assess the various mechanism by which it act.

1. UNCONDITIONED RESPONSE MODELS

Elevated plus maze

EPM is one of the most commonly used model in evaluating the anxiolytic effect of a drug. In a study by Montgomery et al who investigated whether novel exposure would stimulate fear as well as exploratory

drive. He identified that approach avoidance conflict behavior was observed when exposed to novel stimulation, increased fear was seen with elevated alleys than closed alleys, strength of fear decreased with time and after a period of non-exposure the strength of fear recovers.

EPM apparatus consist of two open arms and two closed arms raised above a level from ground and are perpendicular to each other.2 The EPM arms are usually 30 cm long 5 cm wide, elevated about 40-60 cm from the ground level. This test is based on the rodents tendency to avoid elevated places and explore novel environment.2 The number of entries and time spent in open arms are indicative of non-anxious behavior and total number of entries indicate the general locomotion of the rodent.6 The rodent is usually placed in the centre square facing the open or closed arm, and the activity is recorded for 5-10 min.The presence of experimenter in the study can affect the animals behavior, hence video recording with or without the use of software is usually practiced.

Other factors like lighting, handling of animals prior to the test, and housing condition can affect the baseline values. Anxiolytic drugs like benzodiazepines increase the percentage entry and time spent in open arms, further to increase the sensitivity of the model ethological behaviors like head dipping in open arm for detecting nonbenzodiazepines anxiolytics can also be studied.

2 **Elevated T maze and zero maze**

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

Elevated zero maze is a circular platform with two enclosed quadrants and two open quadrants, it is designed in a way to eliminated the central square which is present in traditional EPM and allows the rodent to continuously explore the maze. The time spent in open area and number of entries in open arm is recorded for 5 min. Ethological behavior like head dipping over the edge of the platform and stretched

attend posture can also be included, anxiolytics like diazepam increased the ethological measures and time spent in open quadrants.

3. **Light– dark box**

It is based on the ethological behavior of exploring and retreating from unknown spaces. The advantages of this model is it is simple to perform, no need of prior training of animals and easy to interpret.The apparatus consist of light and dark chambers, which is 44×21×21 cm in dimension one third is dark and two third is open in the top and illuminated by the room light, the two chambers are connected by a shutter which is 13 cm long and 5 cm high.

The preference for dark chamber increases when the animal is anxious. The animal is placed in dark chamber initially to acclimatize. The parameter observed in this model is latency to initial transition into light chamber, this is observed after removing the shutter in the connecting door. The time spent in light chamber can also be recorded, drugs having anxiolytic effect increases the time spent in light chamber.

4. **Open field test**

The apparatus initially used was a raised platform which did not have surrounding walls, later the open field apparatus had surrounging walls of about 10 cm height and the arena with wide range of dimensions, 10×20 cm, 20×40 cm, 40×40 cm, and even 100×100 cm and this is equally divided. The animal is placed on the centre of the arena and test is conducted for 5-10 min.

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

5. **Hole board test**

Hole board exploratory model initially for mice to test the anxiolytic character of the drug. The apparatus is a square board with 16 holes each of them around 3 cm in diameter, or other modifications in this dimensions are also used by researchers, which consist of holes, 4 cm diameter, and arena measuring 68×68 cm. The model is based on the specific animal behavior head dipping which is considered as a measure of neophilia. The anxiety level is inversely proportional to the number of head dipping.

In order to overcome the deficiencies with the open field model, researchers started to use hole board

model to screen the drugs for anxiety, commenting that open field is a measure of fearfulness rather than novelty, rather than novelty, because forcing animal in a closed environment does not always stimulated the exploratory behavior of rather, also increases the anxiety, which is correlated with the increased levels of corticosterone in rodents after exposure. The head dipping behavior which is assessed in this model is considered a valid measure of neophilia.

6. **Social interaction test**

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

7. **Novel object recognition test**

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

8. **Marble burying test**

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

the number of marbles used can affect the outcome of the study. It is seen in few studies that low density bedding showed increase in marble burying.

II. CONTIONED RESPONSE MODELS

1. **Fear conditioning tests**

Social fear and avoidance of social situations represent the main behavioral symptoms of social anxiety disorder (SAD) a disorder that is poorly elucidated and has rather unsatisfactory therapeutic choices. Therefore, animal models are needed to study the underlying cause of the disorder and possible novel treatment possibilities. Fear conditioning models involve the encoding of traumatic memories, representing a psychological stress without physical stimuli.

They have been associated with a vulnerability to phobic fears and other anxiety-related disorders, such as panic disorder (PD), agoraphobia, and posttraumatic stress disorder. In this model, administration of anxiolytic drugs immediately before the pairing of conditioned stimulus (CS) and unconditioned (US) (during the memory acquisition process) affects the formation of conditioned learning. If administration occurs before the re-exposure to CS, it will affect fear and anxiety expression acquired during the conditioning. The drug could also affect extinction of the conditioned response, where a new learning process (that the CS no longer predicts the occurrence of the UCS) occurs after repeated exposure to a CS in the absence of the US.

2. The Geller-Seifter and Vogel conflict tests

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

III. ANIMAL MODELS OF STRESS

Psychological and physical stress models Ideally these models should induce stress in rodents by exposure to physical or physiological stress. These procedures might be used in chronic or acute stress, depending on objectives and parameters chose to evaluate the effect of stress on anxiety. The main stress is

psychological and physical stress. Psychological stress includes neonatal isolation, circadian rhythm change, predator tests. Physical stress includes restraint stress, immobilization stress, electric foot shock stress.

a. PSYCHOSOCIAL STRESS

Predator encounter based models

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

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

Neonatal isolation stress

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

Stress induced by circadian rhythm changes

The circadian rhythm of an organism is an integral component of its homeostatic functioning. It is

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

Stress induced by a noisy stimulus

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

Stress induced hyperthermia

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

More detailed analysis of the neurophysiology of stress generation, the cortical areas involved and biochemical quantification of markers of stress can be done by serial measurements of these values after

a set starting point of evaluation

b. PHYSICAL STRESS

Restraint and immobilisation stress

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

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

Electric foot-shock induced stress

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

Social defeat stress

This model uses a single mouse (intruder) in the home cage of a resident male mouse (aggressor). During this test, behaviors related to confrontation of the intruder by the aggressor are studied. The time spent by an intruder in social defeat posture which is induced by the presence of an aggressor is computed through-out five trials by a blinded observer. Social defeat posture is identified by the following criteria: Immobility (intruder places all four paws on ground, and is oriented towards the aggressor), escape (whereby the intruder tries escaping from the aggressor), crouching (Intruder keeps all four paws on the ground, but isn't oriented towards the aggressor), or defensive upright stance (whereby the intruder stands in an erect fashion with its forepaws extended). This stressor protocol has great relevance in socio-historical context of human ethology (behavior) whereby people show tremendously raised anxiety and alarmed behavior when confronted by a stranger. This rodent model can be studied both in acute instances

of social defeat posturing as well as the behavioral traits exhibited by the intruder upon prolonged/repetitive exposure to the resident aggressor whereby adaptation and diminished levels of anxious behavior is expected. These findings can be further qualified by subjecting the respective animal models to Standard tests and biochemical quantification of markers of anxiety.

Chronic unpredictable stress

This model has been widely used to induce persistent stress-related behavioral changes in rodents. It consists of randomly presenting different stressors to the rodents daily. This prevents the stress-adaptation process which is observed in other models of chronic stress. Here animals are exposed for 2-5 weeks to a wide range of stressors, like foot shocks, restraint stress, light-dark cycle reversal, unpleasant noises, changes in the home cage, heating (37°C) or cooling (4°C) of the home cage, etc. After being exposed to these stressors for several days, the animals exhibit an increased Hypothalamic-Pituitary-Adrenal axis sensitivity and a reduction of responses to pleasant stimuli, however the exploratory behavior is unfazed. This model has good face validity as it represents the stressors faced by human beings in everyday life more realistically. Also, it has an excellent predictive validity, since repeated treatment with antidepressants like fluoxetine, desipramine or imipramine reverses the behavioral effects induced by this model.

OTHER MODELS OF ANXIETY

Grooming analysis algorithm

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

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

PD is chronic and psychiatric disabling disorder featured by unexpected and recurrent attacks and about 5% people of worldwide are affected by this. PD patients psychosocial impairment and have a high risk of psychiatric comorbidities and suicide. The periaqueductal grey matter (PAG) is a midbrain structure that, with other functions, integrates defensive behavior. In humans, electrical stimulation of this evokes

strong feelings of fear, impending death, non-localized pain, and marked autonomic changes. Given the striking similarities between autonomic and behavioral effects of dPAG stimulation and symptoms of panic attacks, it has been suggested that this structure is involved in the genesis of PD in humans and that stimulation of this midbrain area in animals can model panic attacks. Stimulation of dPAG is usually performed in a circular arena (40 cm in diameter) with 40 cm-high walls made of transparent Plexiglas. For chemical stimulation, direct injection of an N-methyl-Daspartate (NMDA) agonist or GABAergic antagonist induces defensive behaviors. For electrical stimulation, a brain electrode is connected to the stimulator by means of an electromechanical swivel and a flexible cable, allowing ample movement of the animal inside the experimental cage. The current is generated by a sine-wave stimulator and monitored on the screen of an oscilloscope. After stimulation of t dPAG, a vigorous reaction is observed, with freezing response, piloerection, miosis, vertical jumps, and a strong flight reactions.

ANTIPARKINSON MODELS

Parkinson's disease is a progressive neurodegenerative illness characterized by tremors, muscular rigidity, bradykinesia, and postural imbalance that worsens over time

- It affects approximately 1% of the population over the age of 65 years
- is regarded as the second most common neurodegenerative disease
- Clinical diagnosis relies on the presence of such features, it is also associated with many non-motor symptoms that add to overall disability
- Also, the imbalance between dopamine and acetylcholine in the Substantia nigra (SN) is a cause of PD. Although characterized as a neuromuscular disorder, dementia, sialorrhoea
- soft speech and difficulty in swallowing due to uncoordinated movements of mouth and throat also occurs at a much greater rate in PD patients over the normal-aged population

It is also reported as the decline in caudate-putamen dopamine content which led to the introduction of dopamine replacement therapy. The etiology of PD remains unknown; several factors appear to play a role, including the aging process, environmental chemicals, oxidative stress, and genetic aspects. A lesion in PD is a marked deficiency in the dopaminergic innervation of the basal ganglia . The discovery that subjects exposed to pyridine derivative, 1 - methyl - 4 - phenyl - 1, 2, 3, 6 - tetrahydropyridine (MPTP), a by-product of synthetic heroin, developed a profound parkinsonian state that led to intense study of the pathogenesis of PD. The primary motor controlrelated symptoms have shown to be a result of

dysregulation of the motor cortex via the nigrostriatal pathway. This dysregulation is caused by the depletion of DA-producing neurons within the pars compacta region of the substantia nigra that project to the striatum. This is often accompanied by Lewy bodies, which are abnormal aggregates of protein that develop inside nerve cells, formed mainly by α-synuclein and ubiquitin.

In-vivo Methods:

1. **Pharmacological Models**

As the name suggests, pharmacological agents induce a parkinsonian state in animals. A drawback of this model is that these are not effective for drugs used for repeated administration.

a) **Tremorine and Oxotremorine Antagonism**:

These agents are muscarine agonists and induce parkinsonism-like symptoms such as tremor, ataxia, spasticity, salivation, lacrimation, and hypothermia. Oxotremorine induces oxidative stress and is implicated as a common pathway in the development of Parkinson's symptoms. They increase reflex and spontaneous activity. An increase in reflex activity is accompanied by rigidity whereas spontaneous activity consists of rhythmic bursts of discharges. Muscarine antagonists are used for reversing the effects. Methodology: Three groups of six male NMRI mice are used. Group A serves as a control. Group B is dosed orally with test compound, whereas group C is given standard (5mg/kg Benzatropine mesylate) 1 h before administration of 0.5mg/kg oxotremorine. Body temperature is noted before administration of test compound (basal value) and after administration at an interval of 1 h for 3 h. This model measures only central anticholinergic activity.

Observation and Evaluation: Hypothermia: The difference in body temperature between intervals is observed and compared with standard and control groups. Tremor: Tremor is scored after oxotremorine injection in a 10s observation period every 15min for 1 h.

b) Reserpine Antagonism:

The reserpine-treated rodent was one of the earliest animal models employed in PD research 11. Reserpine binds to storage vesicles of catecholamines and also blocks VAMT-2 (Vesicular monoamine transporter-2). Thus, it temporarily depletes the storage of dopamine in their respective neurons in the brain. In addition, there is a gradual loss of vesicle stored dopamine as it is used up by release so that the storage vesicles eventually become dysfunctional. The reductant is the depletion of dopamine in neuron 8 . Thus, reserpine is used to induce Parkinson-like symptoms. Reserpine

produces ~85% loss of dopamine in the Substantia nigra and >95% dopamine depletion in the striatum. This is regarded as the fastest method of inducing PD.

Methodology: Male NMRI mice are used. They are injected with 5mg/kg i.p. reserpine. After 24 h animals are tested. The test compound is injected 30 min before observation. The animals are placed onto the floor of a Perspex container and observed.

Observation and Evaluation: Horizontal movements are recorded for 10min. Also rearing, grooming episodes are observed. They are scored according to the severity and are compared using ANOVA

c) Haloperidol Induced Catalepsy:

Haloperidol causes the dysfunction of many neurotransmitters such as acetylcholine, GABA, and serotonin. Primarily used as an anti-psychotic drug, it blocks dopamine receptors in the brain. It functions by disrupting receptors of dopamine D1 and D2 in medium spiny neurons, which include motor circuit indirect and direct pathways. This leads to blockage of striatal dopamine transmission, which causes abnormal downstream firing in the basal ganglia as symptoms of muscle stiffness, locomotive activity, and catalepsy. It induces catalepsy by increasing oxidative stress. It also induces a state in mice/rats in which the animal cannot correct the externally imposed posture (catalepsy)

Methodology: Rats are divided into five groups. Each group contains 6 rats. Catalepsy is induced by injecting 1mg/kg i.p. haloperidol. Group 1 serves as vehicle control. Group 2 serves as standard (levodopa 6mg/kg p.o.). Group 3-5 serves as the test group in which the test compound is injected. The standard bar test is used to measure catalepsy in animals.

Observation and Evaluation: The observations are noted every 30min for 210min. The duration for which the rats retain the four paws extended and resting on the elevated bar was considered as a cataleptic score.

d) Circling Behavior in Nigrostriatal Lesioned Rats:

6-hydroxydopamine (6-OHDA) is a neurotoxin that is used to induce lesions in rats. They act by several mechanisms: formation of free radicals, inhibition of mitochondrial respiratory chain

complexes 1 and 4, inhibition of respiratory enzymes . The rats show a typical behavior of rotating in the direction of lesion i.e., ipsilateral when an indirect-acting compound (amphetamine) is administered and contralateral when a directacting compound is administered. A disadvantage of this method is low sensitivity to small changes in striatal dopamine . This test measures central dopamine function and evaluates the mode of action of novel drugs on dopaminergic neurons.

Methodology: Male Wistar rats are used. The animals are anesthetized with sodium pentobarbital (60mg/kg i.p.). A sagittal cut is made to the skin of the skull. A 2mm wide hole is drilled. Then a 30G Stainless steel cannula connected to a Hamilton syringe is aimed at the anterior zona compacta of the substantia nigra. Then 8mg of 6- OHDA in saline is injected. The wound is closed and allowed to recover. During the recovery time, the development of lesions occurs. The test compound is administered to the animals, and the circling behavior is noted.

Observation and Evaluation:

The number of full turns, ipsilateral or contralateral to the lesions, is recorded every 15 min for 2 h. ED50 values are calculated.

e) **Elevated Body Swing test**:

Borlongan and Sanberg in 1995 proposed this test to measure asymmetrical motor behavior. Here, 6-OHDA is used to induce lesions.

Methodology: 8 week old male Sprague-Dawley rats are used. They are anesthetized with sodium pentobarbital 60mg/kg i.p. and mounted on Kopf stereotaxic frame . 6-OHDA in 4ml saline containing 0.02% ascorbic acid is injected in the left substantia nigra. After 7 days behavioral test is performed. The animal is allowed to attain a neutral position resting its four paws on the ground. The animal is then lifted 2.5cm above the tail. A swing is recorded when the animal moves its head to either side of the central axis.

Observation and Evaluation: Swings are recorded at the interval of 15 sec for 60 sec. The percentage of left and right swings are determined. Two-way ANOVA is used to analyze swing behavior across data.

f) Skilled Paw Reaching in Rats:

6-OHDA is used to induce impairment of paw reaching on both sides. These effects can be reversed by antiparkinsonian drugs or by transplantation of nigral cell suspension.

Methodology: The apparatus used in this test is a narrow Perplex chamber with a lid Fig. 2. A double staircase is placed at the end of the box. Each staircase contains a well, and inside each well 45 mg, saccharin-flavored pellets are kept. Two groups, each containing 6 rats, are used. Group A is kept deprived of food a week before starting the test, whereas group B receives a regular nutrition diet. During this period, they are made familiar with appetitive saccharin-flavored pellets. The animals are placed in the box once per day for 10- 15 min for 4 weeks. The animals are observed and noted. Lesions are induced by injecting 4mg/ml 6-OHDA in 0.9% saline containing 0.01% ascorbic acid. The animals are treated with test compound or saline 30min before 6-OHDA induced lesions.

Observations and Evaluation: The number of pellets eaten during the test period is noted. This indicates the rat's success in grasping and retrieving the pellets. The number of steps from which pellets have been removed is noted. This indicates attempts to reach food and how far the rat can reach. The number of missed pellets is noted. This shows a lack of sensorimotor coordination in grasping and retrieving the pellets. Also, which forepaw is used first by the rat to reach the pellet is noted. All the above parameters are subjected to two-way ANOVA.

g) **Stepping test in Rats**:

This test was introduced by Schallert et al. in 1992 as a relevant model for Parkinsonian akinesia. Lesions were induced by 6- OHDA, which caused impairment in the initiation of stepping movements. In such tests, training and testing are involved, which is time-consuming.

Methodology: Female Sprague Dawley rats are used. They receive two 6-OHDA 3.6mg/ml injections in 0.2mg/ml ascorbate-saline into the right ascending mesostriatal pathway. The experimental setup consists of a smooth-surfaced wooden ramp of 1m in length connected to the rat's cage. The observations are made and the test is performed for three consecutive days. The test is repeated weekly to determine baseline after 6-OHDA lesions. The test drug is administered only once.

Observations and Evaluation: The wooden ramp's initiation time, stepping time, and step length are observed. The data obtained is subjected to ANOVA and Fisher post hoc test.

2. <u>Toxin-induced Rodent Models:</u>

Toxin-induced destruction of the nigrostriatal pathway has proved highly effective in detecting novel

dopaminergic approaches to treatment and avoiding or reversing motor fluctuations and motor complications that occur during therapy as a result of disease progression. These models represent the classic and oldest experimental models of Parkinson's disease. They aim to mimic the disease in humans by the use of certain neurotoxins, which primarily cause lesions in the brain. Neurotoxins such as MPTP and 6-OHDA are commonly used. These models have been established and validated as useful models for the development of therapeutic strategies aimed to treat motor symptoms and to study alterations of the basal ganglia that occur in this disease.

a) MPTP Model in Monkey:

The discovery that MPTP produces PD in humans led to the discovery of the MPTP-induced PD model in primates. It is a commonly used toxin for inducing both rodent and primate models of PD based on its ability to induce persistent Parkinsonism in man. MPTP (N-Methyl4 - phenyl - 1, 2, 3, 6 - tetrahydropyridine) is a mitochondrial complex-1 inhibitor used to induce ymptoms of Parkinson's disease. It induces neurotoxicity in the dopaminergic neurons in mice, rats, cats, dogs, monkeys, and other higher mammals. It causes partial destruction of basal ganglia, which is a major cause of developing PD. They selectively target dopaminergic neurons. MPTP is metabolized by the enzyme MAO-B to 1-methyl-4-phenyl-2,3-dihydropyridine (MPDP+), generating the respective Pyridium species MPP+ and dopaminergic neurons are selectively vulnerable to MPP+ which thus leads to the development of PD. MPTP-treated mice do not systematically show hypokinesia (paucity of movements); unilateral models can have increased activity due to spontaneous rotations. MPTP injected into rats causes only transient Parkinsonian-like symptoms since rats are exceptionally resistant to MPTP

An advantage of this model is that long-term spontaneous compensatory dopaminergic striatal sprouting can be observed. MPTP can be administered acutely or chronically by different routes. Chronic MPTP-induced monkey models of PD also show dopaminergic cell loss, α-synuclein aggregation, α-synuclein upregulation and neuritic α-synuclein pathology. It is found that nicotine protects dopaminergic neurons in the MPTP mouse model, and it also has protective effects in the primate MPTP model and the 6-OHDA-, rotenone-, and paraquat-induced animal models of PD. MPTP easily crosses the blood-brain barrier because it is a lipophilic molecule where it is metabolized to MPP^- by non-neural cells . It is an effective model for repeated drug evaluation

Methodology: Eight adult rhesus monkeys are injected with a cumulative dose of N-MPTP up to 10-

18mg/kg i.v. for a period of 5-8days. They show parkinsonism-like symptoms. The test compound is administered and observed for reversal of symptoms. Observations and Evaluation: The symptoms are scored based on their severity.

Assessment	Score	Description
1. To assess movements	0	Normal
	1	Reduced
	2	Sleepy
2. To check movements	0	Present
	1	Reduced
	2	Absent
3. To check attention and blinking	0	Normal
	1	Abnormal
4. To check posture	0	Normal
	1	Abnormal trunk
	2	Abnormal trunk and tail
	3	Abnormal trunk, tail, and limbs
	4	Flexed posture
5. To check balance and coordination	0	Normal
	1	Impaired
	2	Unstable
	3	Falls
6. To check reactions	0	Normal
	1	Reduced
	2	Slow
	3	Absent

b) **6-hydroxydopamine Lesioned Rat Model**:

6- hydroxydopamine (6-OHDA) is an analogue of dopamine, and norepinephrine is also being increasingly used with genetically modified mice. It is the first chemical to be found which induces PD in rodents. This is a prototypical model involving the use of 6-OHDA, which when injected locally (Intracerebral) produces neurotoxicity. 6- OHDA does not penetrate the blood brain barrier; hence, direct

administration into the brain parenchyma is required. It is accounted to be the first animal model ever generated, as 6-OHDA was the first compound discovered to induce selective catecholaminergic cell death 10. They act by several mechanisms: formation of free radicals, inhibition of mitochondrial respiratory chain complexes 1 and 4, inhibition of respiratory enzymes. Stereotaxic injection of 6-OHDA into the Substantia nigra pars compacta or the striatum induces neuronal cell death of the tyrosine hydroxylase (TH)-containing neurons in rat and mouse brain, which decreases the dopamine levels in the TH-positive terminals of the striatum. This procedure grants the highest amount of dopamine depletion within 2-3 days. This model is also widely used to evaluate the integrity of the nigrostriatal system and postsynaptic supersensitivity. 6-OHDA has been found in the urine of L-DOPA-treated patients with PD, suggesting that 6-OHDA may play a role in the pathogenesis of PD as an endogenous hydroxylated metabolite of dopamine

Methodology: Rats are divided into six groups, and each group contains six animals. They are anesthetized with an i.p injection of 50mg/kg sodium pentobarbital and fixed in a stereotaxic apparatus. Stainless steel needle is inserted unilaterally in the substantia nigra, and an injection of 6-OHDA is made over 5min and the needle was left in place for a further 5min. The wound area is covered, and the animal is allowed to recover. The treatment of animals is started after 48 h of 6- OHDA induction once a day for 55 days. Group 1 receives normal saline 10ml/kg p.o. Group 2 serves as 6-OHDA control and receives normal saline 10ml/kg p.o. Group 3 serves as standard (levodopa 6mg/kg p.o.). Group 4-6 receives test compound.

Observation and Evaluation: Behavioral assessment of all the six groups is performed. Locomotor activity is evaluated by using a digital actophotometer.

c) **Lipopolysaccharide-induced Model**:

Recent advances in the pathogenesis of PD states that neuroinflammation is a major mediator in the initiation of the disease. This led to various animal model studies that use bacterial endotoxin lipopolysaccharide (LPS) to induce PD. It causes intense tissue inflammation and is directly infused into the nigrostriatal pathway of rats. When injected via the Intranigral route, it results in activation of microglia and degeneration of the dopaminergic system. Lipopolysaccharide is itself not a neurotoxin, but the cytotoxins secreted by them possess the potential for developing the disease. In addition, LPS causes the accumulation of α-synuclein and ubiquitin in neurons. Also, they cause marked rotational behavior, ipsilateral to the lesioned side in response to systemic administration of amphetamine.

Methodology: Rats/mice of either sex are used. The animals are divided into three groups. Group A serves as a control. Group B receives a standard drug (Levodopa). Group C receives test compounds to be screened. Intrastriatal administration of 30 μg of LPS causes a reduction in the number of TH positive cells in SN. The observations are made up to 4 weeks of LPS administration. The rotarod test is performed every week for four weeks, and a reduction in time spent on rotarod is noted. It is compared to standard and control groups

3. **Pesticide-induced Model**: Various pesticides are used as neurotoxins for inducing PD into animals. Both rotenone and paraquat act as inhibitors of the complex I component of the mitochondrial respiratory chain.

a) **Rotenone Induced Model**: Rotenone is a naturally occurring compound that impairs oxidative phosphorylation in the mitochondria by inhibiting reduced nicotinamide adenine dinucleotide (NADH)-ubiquinone reductase activity. Similar to MPTP, the insecticide rotenone is highly lipophilic, so it readily crosses the blood brain barrier and diffuses into neurons where, like MPTP, it accumulates within mitochondria and inhibits complex I. The production of Reactive Oxygen Species (ROS) is thought to induce oxidative stress . The Rotenone model shows various symptoms of PD, such as complex I blockade of mitochondrial function, behavioral dysfunctions, inflammation, synuclein aggregation, Lewy body-like formations, and oxidative stress. However, because of the difficulties associated with using rotenone to generate a model of PD, limited data have been reported 11. It is both a herbicide and insecticide from Leguminosaplants, having a half-life of 3-5 days. Methodology: The animals are divided into four groups, each containing 6 animals. Group 1 serves as a control. Group 2 is administered rotenone for 35 days. Group 3 and 4 receive test drugs for 35 days. Observation and Evaluation: After 24 h of the last dose, behavioural studies are performed. Histological, biochemical analyses are done. Neurochemical studies are also done.

b) **Paraquat Induced Model:**

It is known that paraquat exerts its deleterious effects through oxidative stress and its toxicity through cellular redox cycling. Paraquat, chemically known as 1,1′- dimethyl-4,4′-bipyridinium is used widely as an herbicide, and it exhibits a structural resemblance to MPP+. However, unlike MPP+, paraquat exerts deleterious effects on dopaminergic neurons through oxidative stress-mediated damage of lipids,

proteins, DNA, and RNA by generating reactive oxidative species, superoxide radicals, hydrogen peroxide, and hydroxyl radicals in mice. Paraquat enters the brain via neutral amino acid transporter. Once inside the brain, it induces both indirect neuronal toxicity as well as direct inhibition of mitochondrial complex 1. The latter effect usually occurs at high doses. Paraquat reduces motor activity and induces a dose-dependent loss of striatal tyrosine hydroxylase (TH)-positive neurons of mice. In addition, paraquat induces an increase in α-synuclein and the c) Maneb Induced Model: Maneb is a fungicide manganese ethylene-bis-dithiocarbamate that has been associated with an increased incidence of PD . Attempts have been made to model PD using this agent. Maneb enters the brain and causes inhibition of complex III of the mitochondrial respiratory chain. When combined with paraquat, it is shown to produce enhanced toxicity. d) Methamphetamine Induced Models: Methamphetamine has neurotoxic effects on the nervous system that cause functional deficits and structural alterations. However, although selective dopaminergic or serotonergic neuronal cell loss occurs in rodents following the administration of high doses of methamphetamine, this model is not very reliable; the results only produce a long-term loss of TH enzyme but are not examined in the PDdependent behavioral tests.

NOOTROPICS MODELS

Nootropics are also referred as smart drugs, memory enhancers, and cognitive enhancers. They are reported to improve mental function such as cognition, memory, intelligence, motivation, attention and concentration. They are thought to be work by altering the availability of brains supply of neurochemicals, by improving the brains oxygen supply or by stimulating nerve growth . The concept and definition of a ―Nootropic drug‖ was first proposed by Romanian Dr. Corneliu e. Giurgea. He derived the term nootropics from the Greek words ―noos‖ (=mind) and ―tropein‖ (=to turn towards). The main features of nootropics drug are, the enhancement, at least under same conditions of learning acquisition as well as resistance of learned behaviors to agents that tend to impair them, the facilitation of inter hemispheric flow of information, partial enhancement of the general resistance of the brain and particularly its resistance to physical and chemical injuries and increase in the efficacy of the tonic cortico sub cortical control mechanisms.

PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE

Generally nootropics are used to treat dementia and various forms of dementia. The most common form of the dementia is Alzheimer Disease (AD). This incurable, degenerative and terminal disease is usually confirmed with behavioral assessments and cognitive test often followed by brain scan. As disease

advances symptoms include confusion, irritability, and aggression, mood swings, language breakdown, and long term memory loss. Gradually body functions are lost, leading to death. The duration of disease varies. It developes for an indeterminate period and may remain undiagnosed for many years.

Neuropathology

Alzheimer's disease is characterized by loss of neurons and synapses in the cerebral cortex and certain subcortical regions. This loss results in gross atrophy of the affected regions, including degeneration in temporal lobe and parietal lobe and parts of the frontal cortex and cingulated gyrus. Studies using MRI and PET have documented reduction in the size of specific brain regions in patients as they progressed from mild cognitive impairment to AD.

PRECLINICAL EVALUATION TECHNIQUES FOR NOOTROPICS

IN VITRO METHODS

In vitro inhibition of acetylcholine-esterase activity in rat striatum

Principle : The purpose of this assay is to screen drugs for inhibition of acetylcholine-esterase activity. Inhibitors of this enzyme may be useful for the treatment of Alzheimer's disease. Acetylcholinesterase (AChE), is found in nerve cells, skeletal muscle, smooth muscle, various glands and red blood cells. It is generally accepted that the physiological role of AChE is the rapid hydrolysis and inactivation of acetylcholine. Inhibitors of AChE show marked cholinomimetic effects in cholinergically innervated effector organs and have been used therapeutically in the treatment of glaucoma, myasthenia gravis and paralytic ileus. However, recent studies have suggested that AChE inhibitors may also be beneficial in the treatment of Alzheimer's dementia.

Procedure

Tissue preparation: Male Wistar rats are decapitated, brains rapidly removed, corpora striata dissected free, weighed and homogenized in 19 volumes (approximately 7 mg protein/ml) of 0.05 M NaH2PO4, pH 7.2 using a Potter-Elvejhem homogenizer. A 25 µl aliquot of this suspension is added to 1 ml of the vehicle or various concentrations of the test drug and reincubated for 10 min at 37 °C. Assay: Enzyme activity is measured with the Beckman DU-50 spectrophotometer. This method can be used for IC50 determinations and for measuring kinetic constants. Reagents are added to the blank and sample cuvettes as follows: Blank: 0.8 ml PO4 buffer/DTNB 0.8 ml buffer/Substrate Control: 0.8 ml PO4 buffer/DTNB/Enzyme 0.8 ml PO4 buffer/Substrate Drug: 0.8 ml PO4 buffer/DTNB/Drug/Enzyme 0.8 ml PO4 buffer/Substrate Blank values are determined for each run to control for non-enzymatic hydrolysis of substrate and these values are automatically subtracted by the kindata program available on

kinetics soft-pac module. This program (Beckman DU-50 series spectrophotometer, kinetics Soft-PacTM module operation instructions: 1–7 also calculates the rate of absorbance change for each cuvette. Evaluation: For IC50 determinations: Substrate concentration is 10 mM diluted 1 : 2 in an assay yielding a final concentration of 5 mM. DTNB concentration is 0.5 mM yielding 0.25 mM final concentration. % Inhibition = (slope control-slope drug/slope control)×100 IC50 values are calculated from log probit analysis

Ex vivo cholinesterase inhibition

Principle

This assay is used to determine the doseresponse relationship and duration of action of cholinesterase inhibitors in vivo. Cholinesterase inhibitors, including physostigmine and tacrine have been shown to improve cognitive functions in Alzheimer's disease. Physostigmine is a potent, but nonselective inhibitor of cholinesterase and has a short duration of action. Tacrine also inhibits both acetylcholine- esterase (true) and butyrylcholineesterase (pseudo), but is more potent as an inhibitor of the pseudo-enzyme. Physostigmine is a competitive inhibitor and blocks the active site of the enzyme by carbamylation of a serine hydroxyl group at the esteratic site of the enzyme. This covalently bound carbamyl group then dissociates from the enzyme much more slowly than the acetyl group left by the natural substrate, but the inhibition is not irreversible like that of the organophosphates. The inhibition characteristics of physostigmine, i.e., sub micromolar affinity for the enzyme and covalent binding of the inhibiting group, are ideal for ex vivo studies. Tacrine, however, is a mixed competitive inhibitor of cholinesterase, with lower apparent affinity than physostigmine for the enzyme. Tacrine binds to the anionic site of cholinesterase through weak hydrophobic interactions.

Procedure

Drug treatment: Groups of four male Wistar rats are dosed i.p. or p.o. with vehicle or the test drug. For the initial dose response study, the rats are given varying doses of drug based on toxicity reported in primary overt effects testing and sacrificed at either 30 min or 1 h after dosing. The animals are observed and the occurrence of cholinergic signs is noted (piloerection, tremors, convulsions, salivation, diarrhea and chromodacryorrhea). Tissue preparation: Male Wistar rats are decapitated, brains rapidly removed, corpora striata dissected free, weighed and homogenized in 4 volumes of 0.05 M phosphate buffer, pH 7.2 using a Potter-Elvehjem homogenizer. A 12.5 ml aliquot of the homogenate is added to 1 ml 0.05 M phosphate buffer, pH 7.2/DTNB (reagent 2). Assay 1. Enzyme activity is measured with the Beckman DU- 50 spectrophotometer. Reagents are added to the blank and sample cuvettes as follows: Blank: 0.8

ml PO4 buffer/DTNB (reagent 2) 0.8 ml PO4 buffer/Substrate (reagent 3) Control: 0.8 ml PO4 buffer/DTNB/Enzyme from control animal 0.8 ml PO4 buffer/Substrate Drug: 0.8 ml PO4 buffer/DTNB/Enzyme from treated animal 0.8 ml PO4 buffer/Substrate Blank values are determined for each run to control for non-enzymatic hydrolysis of substrate and these values are automatically subtracted by the kindata program available on kinetics soft-pac module. This program also calculates the rate of absorbance change for each cuvette. 2. Substrate concentration is 10 mM diluted 1: 2 in the assay yielding final concentration of 5 mM. DTNB concentration is 0.5 mM yielding 0.25 mM final concentration.

Evaluation The percent inhibition at each dose or time is calculated by comparison with the enzyme activity of the vehicle control group. %inhibition = (slope control-slope drug/slope control) × 100

Enzyme inhibition studies

For the enzyme inhibition studies, 25 µl aliquots of the enzyme preparation are preincubated with varying concentrations of the inhibitor for 10 min at 25 °C and acetylcholinesterase activity is determined as previously described. Evaluation: Values for the IC50 are determined by log-probit analysis of the inhibition data using six to seven concentrations of the inhibitor and represent the means of 3 separate experiments.

Release of [3H]ACh and other transmitters from rat brain slices

Principle

Electrically stimulated release of [3H] ACh is used as a biochemical screen for agents which may possibly enhance or inhibit release of [3H]ACh through a direct muscarinic interaction or other indirect interactions. Muscarinic autoreceptors have been shown to have a role in the regulation of ACh release in several areas of the CNS. Direct stimulation of muscarinic receptors with muscarinic agonists or indirect stimulation with acetylcholinesterase inhibitors decreases ACh release evoked by either increased potassium concentration or electrical stimulation. Muscarinic antagonists can either block their inhibition, or, under certain conditions, enhance ACh release. This technique measures only presynaptic effects of test compounds.

[3H] Oxotremorine-m binding to muscarinic cholinergic receptors in rat forebrain

Principle

The muscarinic receptors are members of the super family of G-protein-coupled receptors. They are relatively abundant and mediate the diverse action of acetylcholine in the CNS, as well as throughout non-nervous tissues innervated by the parasympathetic nervous system. The purpose of this assay is to determine the binding affinity of potential cholinomimetic drugs for muscarinic receptors in brain, using an agonist ligand. Oxotremorine is a potent centrally and peripherally acting muscarinic cholinergic agonist, which has been shown to be active in isolated tissue preparations as well as in vivo. Both central and peripheral effects of oxotremorine are blocked by antimuscarinic drugs such as atropine. Structural modification of the oxotremorine molecular yields compounds which are full agonists, partial agonists and antagonists at muscarinic receptors. Oxotremorine-M (oxo-M), a quaternary nitrogen analog of oxotremorine, is a full agonist for the phosphatidyl-inositol response. Oxotremorine and oxo-M are full agonists for inhibition of adenylate cyclase of the muscarinic agonists, oxotremorine is the most potent inhibitor of [3H] QNB binding, and however, the IC50 is still only in the micromolar range. The apparent low affinity of agonist competition for [3H]-antagonist.

INVIVO METHODS

Inhibitory (passive) avoidance

One of the most common animal tests in memory research is the inhibition to imitate activities or learned habits. The term ―passive avoidance‖ is usually employed to describe experiments in which the animal learns to avoid a noxious event by suppressing a particular behavior.

1. **Step-down**

Principle : An animal (mouse or rat) in an open field spends most of the time close to the walls and in the corners. When placed on an elevated platform in the center of a rectangular compartment, it steps down almost immediately to the floor to explore the enclosure and to approach the wall.

Procedure: Mice or rats of either sex are used. A rectangular box (50 x 50 cm) with electrifiable grid floor and 35 cm fits over the block. The grid floor is connected to a shock device which delivers scrambled foot shocks. The actual experiments can be performed in different ways. A typical paradigm consists of three phases: (1.) Familiarization: The animal is placed on the platform, released after raising the cylinder, and the latency to descend is measured. After 10 s of exploration, it is returned to the home cage. (2.) Learning: Immediately after the animal has descended from the platform an unavoidable footshock is applied (Footshock: 50 Hz; 1.5 mA; 1 s) and the animal is returned to the home cage, (3.) Retention Test: 24 h after the learning trial the animal is again placed on the platform and the step-down latency is

measured. The test is finished when the animal steps down or remains on the platform (cut-off time: 60 s).

Evaluation: The time of descent during the learning phase and the time during the retention test is measured. A prolongation of the step-down latency is defined as learning. The variability of this method is relative high; therefore, it is necessary to test large groups of animals (minimum 10 animals per group).

2. **Step-through**

Principle: This test uses normal behavior of mice and rats. These animals avoid bright light and prefer dim illumination. When placed into a brightly illuminated space connected to a dark enclosure, they rapidly enter the dark compartment and remain there. The standard technique was developed for mice and modified for rats. It is widely used in testing the effects of memory active compounds.

Procedure: Mice and rats of either sex are used. The test apparatus consists of a small chamber connected to a larger dark chamber via a guillotine door. The small chamber is illuminated with a 7 W/12 V bulb. The test animals are given an acquisition trial followed by a retention trial 24 h later. In the acquisition trial the animal is placed in the illuminated compartment at a maximal distance from the guillotine door, and the latency to enter the dark compartment is measured. Animals that do not step through the door within a cut-off time: 90 s (mice) or 180 s (rats) are not used. Immediately after the animal enters the dark compartment, the door is shut automatically and an unavoidable footshock (Footshock: 1 mA; 1 s – mice; 1.5 mA; 2 s – rat) is delivered. The animal is then quickly removed (within 10 s) from the apparatus and put back into its home cage. The test procedure is repeated with or without drug. The cut-off time on day 2 is 300 s (mice) or 600 s (rats), respectively.

Evaluation: The time to step-through during the learning phase is measured and the time during the retention test is measured. In this test a prolongation of the step-through latencies is specific to the experimental situation. An increase of the stepthrough latency is defined as learning

3. **Two compartment test**

Principle: A rodent in an open field tends to enter any recesses in the walls and to hide there. When placed into a large box, connected through a narrow opening with a small dark compartment, the animal rapidly finds the entrance into the small chamber, enters it and spends most of its time there. The times spent in the large and small compartments are measured. The latency of the first entrance into the dark chamber and the number of crossings from one compartment into the other can be used as auxiliary criteria.

Procedure: Mice and rats of both sex and a rectangular box with a 50 x 50 cm grid floor and 35 cm high walls are used. In the centre of one wall is a 6 x 6 cm opening connecting the large compartment to a

small 15 x 15 cm box with dark walls, electrifiable grid floor and removable ceiling. The connection between the two compartments can be closed with a transparent sliding door. Illumination is provided with a 100 W bulb placed 150 cm above the centre of the large compartment.

Evaluation: The times the animal spends in the large and the small compartment are measured.

4. **Up-hill avoidance**

Principle: Many animal species exhibit a negative geotaxis, i.e. the tendency to orient and move towards the top when placed on a slanted surface. When placed on a tilted platform with head facing downhill, rats and mice invariably turn around and move rapidly up the incline.

Procedure: Rats of both sex were used and maintained under standard conditions. The experimental apparatus is a 50 x 50 cm box with 35 cm high opaque plastic walls. The box can be inclined at different angles. The floor consists of 10 mm diameter stainless steel grid bars placed 13 mm apart. To deliver the tail-shock, a tail electrode is constructed, consisting of a wire clip connected to a constant current shock source. The animal is first fitted with the tail-electrode and then placed onto the grid with its nose facing down. During baseline trials the animal's latency to make a 180° turn and initiate the first climbing response is measured. Thereafter the animal is returned to its home cage. During the experimental trials the latencies are measured and additionally a tail-shock (1.5 or 2 mA) was administered contingent on the first climbing response after the 180° turn. Immediately after the shock the animal is placed in its home cage. Retest is performed 24 h later.

Evaluation: The latencies are measured.

5. **Trial-to-criteria inhibitory avoidance**

Principle: As animals experience different sensitivity to the footshock punishment applied in the dark area, immediately after the first trial the animal is returned to the lighted area to evaluate if the task has been acquired. A criteria is established to determine the learning of the test, usually requiring the animal to remain in the lighted area for a period of 30–60 s. In this way, all the animals have a similar degree of learning independently of the amount of trials needed to attain it.

Procedure: Mice or rats are generally used. The animals are trained in the same way as in the stepthrough version. They are placed in the lighted compartment and after they entered with the four paws into the dark area, the door is closed and a mild footshock is delivered. Immediately after the shock they are placed back in the lighted area for another trial. Training would continue this way until the animal remains in the lighted area for a certain period of time (30 or 60 s), a time at which the training is considered to

be acquired by all the animals. The numbers of trials to attain criteria are counted as an indication of the speed of acquisition.

Evaluation: Retention of the test is measured 24 or 48 Hs later. The animals are placed in the lighted area, the door opened and the latency to step with the four paws into the dark area is recorded. A cut-off latency of 180 or 300 s is usually imposed.

6. **Scopolamine induced amnesia in mice**

Principle: The administration of the antimuscarinic agent scopolamine to young human volunteers produces transient memory deficits. Analogously, scopolamine has been shown to impair memory retention when given to mice shortly before training in a dark avoidance task. The ability of a range of different cholinergic agonist drugs to reverse the amnesic effects of scopolamine is now well documented in animals and human volunteers. However, the neuropathology of dementia of the Alzheimer type is not confined to the cholinergic system.

Procedure: The scopolamine test is performed in groups of 10 male NMRI mice weighing 26–32 g in a one-trial, passive avoidance paradigm. Five min after i.p. administration of 3 mg/kg scopolamine hydrobromide, each mouse is individually placed in the bright part of a two-chambered apparatus for training. After a brief orientation period, the mouse enters the second, darker chamber. Once inside the second chamber, the door is closed which prevents the mouse from escaping, and a 1 mA, 1-s foot shock is applied through the grid floor. The mouse is then returned to the home cage. Twenty four hours later, testing is performed by placing the animal again in the bright chamber. The latency in entering the second darker chamber within a 5 min test session is measured electronically. Whereas untreated control animals enter the darker chamber in the second trial with a latency of about 250 s, treatment with scopolamine reduces the latency to 50s. The test compounds are administered 90 min before training. A prolonged latency indicates that the animal remembers that it has been punished and, therefore, does avoid the darker chamber.

Evaluation: Using various doses latencies after treatment with test compounds are expressed as percentage of latencies in mice treated with scopolamine only. In some cases, straight dose response curves can be established whereas with other drugs inverse U-shaped dose-responses are observed.

7. **Memory impairment by basal forebrain lesions in rats**

Principle: Memory impairment can be produced by lesions caused by bilateral injections of ibotenic acid into the basal forebrain of rats. Water maze tasks, habituation tasks, passive avoidance tasks with a

light/dark compartment apparatus, and the inhibition of the decrease of choline acetyltransferase activity in the cortex can be used to evaluate the effect of drugs.

Procedure: Male Wistar rats weighing 270–310 g are anesthetized with sodium pentobarbital (45 mg/kg i.p.) and placed in a stereotaxic apparatus. Neurotoxic lesions of the basal forebrain are produced by injection of ibotenic acid. An injection needle connected to a 5-µl microsyringe is inserted into the basal forebrain. Ibotenic acid is dissolved in 50 mM Na phosphate buffer at a concentration of 12 µg/ml, and then 0.5 ml (6 µg per side) is infused for 5 min. The injection needle is left in place for an additional 5 min to allow the toxin to diffuse away from the needle tip. One week later, the contralateral side is treated in the same manner. The same procedure is used to administer microinjections of 50 mM Na phosphate buffer into the basal forebrain of shamoperated rats. Three to 5 weeks after the first lesion, the animals are tested on the acquisition of a task in a Morris water maze (Morris 1981), on a habituation task in a novel situation, and in a passive avoidance task with light and dark compartments. The rats are treated once a day during the experiment. After the behavioral experiments, the animals are sacrificed for determination of choline acetyltransferase activity in the brain. The tissue is homogenized (4% w/v) in cold 50 mM Na phosphate buffer (pH 7.4), and Triton X-100 (0.55, v/v) is added to homogenates to ensure enzyme release. To 75 µl of enzyme solution, 125 µl of substrate mixture (0.4 mM [14C]acetyl-Co A (50.6 mCi/mmol), 300 mM mM EDTA-2Na, and 0.1 mM physostigmine) is added in a scintillation vial and the mixture is incubated at 37 °C for 30 min. After the incubation, 0.8 ml of cold 50 mM phosphate buffer, 0.5 ml of acetonitrile containing 2.5 mg of tetraphenylborate and 2.0 ml toluene are added to the scintillation vial. The vials are shaken lightly and allowed to stand overnight before radioactivity is determined.

Evaluation: Data are evaluated by usual statistical means. All analyses are followed by a Bonferroni's test. NaCl, 50 mM Na phosphate buffer (pH 7.4), 8 mM choline chloride.

8. **Ischemia induced amnesia in gerbils**

Principle: Impairment of cerebral metabolism induced by reduced blood supply is known to induce cognitive deficits. Because of the absence of posterior communicating arteries in the brain of Mongolian gerbils, complete forebrain ischemia can be produced by occluding both common carotid arteries resulting in amnesia.

Procedure: Male Mongolian gerbils weighing 50–70 g are anesthetized by i.p. pentobarbital injection. Both common carotid arteries are exposed through a ventral neck incision and occluded for 5 or 10 min

with miniature aneurysm clips. In sham-operated controls, the common carotid arteries are exposed but not occluded. Twenty-four hours after occlusion, each animal is placed in the bright part of a light/dark-chambered apparatus for training. After a brief orientation period, the gerbil enters the second, dark chamber. Once inside the second chamber, the door is closed which prevents the animal from escaping, and a 100 V, 2sec foot shock is applied through the grid floor. The gerbil is then returned to the home cage. Twenty-four hours later, testing is performed by placing the animal again in the bright chamber. The latency in entering the second dark chamber within a 5 min test session is measured electronically. The latency compared with shamoperated controls is decreased depending on the duration of ischemia. After drug treatment, an increase of latency in entering the dark compartment indicates good acquisition.

Evaluation: Using various doses a dose-dependent increase of latency can be found after active drugs, sometimes resulting in inverse U-shaped doseresponse curves.

ACTIVE AVOIDANCE

Active avoidance learning is a fundamental behavioral phenomenon. As in other instrumental conditioning paradigms the animal learns to controlthe administration of the unconditioned stimulus by appropriate reactions to the conditioned stimulus preceding the noxious stimulus. The first stage of avoidance learning is usually escaped, whereby a reaction terminates the unconditioned stimulus.

1. **Runway avoidance**

Principle: A straightforward avoidance situation features a fixed aversive gradient which can be traversed by the animal. The shock can be avoided when the safe area is reached within the time allocated.

Procedure: Mice or rats of either sex are used and maintained under standard conditions and handled for several days before the experiment. The same box as used in the step-through model can be used in this experiment. The apparatus is uniformly illuminated by an overhead light source. A loudspeaker, mounted 50 cm above the start-box, serves for presenting the acoustic conditioned stimulus. The footshock is employed by the same source as in the step-through avoidance. The animal is allowed to explore the whole apparatus for 5 min. The guillotine door is then closed and the animal is placed into the light starting area. After 10 s the acoustic CS is applied and the door is simultaneously opened. Shock is turned on after 5 s. The CS continuous until the animal reaches the safe area. It is left there for 50–70 s before returned to the same area again. The procedure starts again. The training is continued until the animal attains the criterion of 9 avoidances in 10 consecutive trials. On the next day the procedure is repeated until the same learning criterion is reached. The time needed to reach the safe area is measured.

Evaluation: The time the animal needs to reach the safe area on both days is measured. In addition, the number of errors (not reaching the safe area) is recorded.

2. **Shuttle box avoidance (two- way shuttle box)**

Principle: Compared to runway avoidance, shuttle box avoidance (two-way-shuttle-box) is a more difficult task. Since the animal is not handled between trials, the shuttlebox can be easily automated.

Procedure: Rats of both sexes are used and maintained under standard conditions.

The apparatus used consists of a rectangular box 50 × 15 cm with 40 cm high metal walls, and an electrifiable grid floor. The box is divided by a wall with a manually or solenoid-operated guillotine door (10 × 10 cm) into two 25 × 15 cm compartments. Each compartment can be illuminated by a 20 W bulb mounted in thehinged Plexiglas lids. A fixed resistance shock source with an automatic switch (0.5 s on 1.5 s off) is used. Simple programming equipment provides for automatic delivery of the conditioned stimulus (CS) and the unconditioned stimulus (US). The apparatus is placed in a dimly light room with a masking noise background (white noise) of 60 dB. The animal is allowed to explore the apparatus for 5 min with the connecting door open and the compartment lights switched off. The guillotine door is then closed. After 20 s the light is switched on in the compartment containing the animal, and the door is opened. A tone (CS) is presented and 5 s later the floor shock is applied in the illuminated compartment and continued until the animal escapes to the dark side of the compartment, the connecting door is closed and the shock discontinued. After a variable inter trial interval (30–90 s) the light is switched on in the previous dark compartment, the door is opened and the animal is required to cross to the other side. The training is continued until the animal reaches the criterion of 9 avoidances in 10 consecutive trials. Retention is tested at different intervals after the original training by retraining the animal to the same criterion again.

Evaluation: The time the animal needs to reach the safe area on both days is measured. In addition, the number of errors (not reaching the safe area) is recorded.

3. **Jumping avoidance (one-way shuttle box)**

Principle: Since a high degree of automation and minimum handling are additional requirements for this model, the obvious solution is a simplified oneway avoidance, allowing for the spontaneous or forced return of the animal to the start. In order to enhance the start-goal distinction a vertical gradient is introduced which requires the animal to perform a discrete response of an all-or-none character, such as

the jump, which clearly differs from the more continuous translational movements required in the usual avoidance tasks.

Procedure: Rats of both sex are used and maintained under standard conditions. The apparatus used consists of a rectangular box 40 × 25 cm with 40 cm high metal walls, an electrifiable grid floor and a Plexiglas ceiling. A 12 × 12 × 25 cm opaque plastic pedestrial, mounted onto one of the narrow walls of the box provides the isolated goal area. Flush with the horizontal surface of the pedestal moves a vertical barrier, which can either be retracted to the rear wall of the apparatus to expose the goal area or pushed forward to block access to the goal completely. The animal is placed into the apparatus for 5 min with the goal area exposed (barrier re-tracted). The barrier is then moved forwards and the goal is blocked for 2 s. The first trial starts by exposing the goal area and applying an acoustic CS (1 000 Hz, 85 dB). Electric shocks – US (1.0 mA; 50 Hz; 0.5 s) – are applied 5 s later (once per 2 s), and continued together with the CS until the animal jumps onto the platform. After 30 s the barrier pushes the animal off the platform onto the grid floor. The sequence is repeated until the criterion of 10 consecutive avoidances is reached. Retention is tested on the next day until the animal reaches criterion.

Evaluation: The time the animal needs to reach the safe area on both days is measured. In addition, the number of errors is recorded.

Discrimination learning

In the experiments described above the animals have no choice between the conditioned stimuli. They have only one conditioned stimulus. The following examples illustrate the special techniques employed for discrimination among different stimulus modalities. The experiments can be classified either as simultaneous or successive discrimination paradigms.

1. **Spatial habituation learning**

Principle:The open-field test utilizes the natural tendency of rodents to explore novel environments in order to open up new nutrition, reproduction and lodging resources. The rate of exploratory behaviors exhibited in an unfamiliar environment is limited through the inherent necessity to avoid potential dangers. The observed behavior therefore is always a compromise between these conflicting interests and is regulated in part by the momentary physiological needs. Spatial habituation learning is defined as a decrement in reactivity to a novel environment after repeated exposure to that now familiar environment. This reduction in exploratory behaviors during reexposures is interpreted in terms of remembering or recognition of the specific physical characteristics of the environment. The test can be used to examine

short-term spatial memory and/or long-term spatial memory.

Procedure: The open-field apparatus is a rectangular chamber (rats: 60 × 60 × 40 cm, mice: 26 × 26 × 40) made of painted wood or grey PVC. A 25 W red or green light bulb is placed either directly above or beneath the maze to achieve an illumination density at the centre of approximately 0.3 lx. Masking noise is provided by a broad spectrum noise generator (60 dB). Prior to each trial, the apparatus is swept out with water containing 0.1% acetic acid. Housing room and the testing location are separated and animals are transported to the testing room 30 min before testing. The digitized image of the path taken by each animal is stored and analyzed post hoc with a semi-automated analysis system. In aged or hypoactive rodents testing is performed during the animal's dark phase of day. The rodent is placed on the center or in a corner of the open-field for 5–10 minute sessions. The animals are re-exposed to the open-field 24 and 96 h after the initial trial.

Evaluation: The exploratory behaviors' registered are: (1) Rearing or vertical activity: the number of times an animal was standing on its hind legs with forelegs in the air or against the wall. (2) The duration of single rearing as a measure of nonselective attention Locomotion or horizontal activity: the distance in centimeters an animal moved.

2. **Spatial discrimination**

Principle: In the simplest case of discrimination learning the animal distinguishes between two symmetric stimulus response sets, the equal probability of which has been changes by differential reinforcement events. Position of the cues with respect to the animal's body defines the CS+ and CS– . Usually left-right discrimination is employed, while axial orientation of the body is ensured by the construction of the apparatus.

Procedure: Rats and mice of both sexes are used and maintained under standard conditions. The apparatus used is usually a simple T- or Y-maze, with an electrifiable grid floor. The last 10 cm of each arm are separated from the rest of the apparatus by a swing-door which prevents the animal from seeing the food cup or the plastic sheet covering the grid in the goal area. A fixed resistance shock source is connected to an automatically operated switch. In an aversively motivated spatial discrimination learning the animal is trained to escape and/or to avoid foot shocks by always going to the right. Training starts by allowing the animal to explore the apparatus. Then the animal is placed on the start and after 5 s electric shocks (0.5 s, 50 Hz, 1.0 mA) are applied at 3 s intervals. The animals are trained to a criterion. On the following day the animal is retrained to the same criterion. After a 60 min interval the safe goal area is shifted to the other arm of the maze and the discrimination is reversed.

Evaluation: Errors are scored. An error means that the animal enters the wrong arm with all four legs. During retention the number of trials until the animal makes correct choices is counted.

3. **Spatial learning in the radial arm maze**

Principle: Olton and co-workers have developed a spatial discrimination task for rodents that has been extensively used in learning and memory studies, and that has served as the basic task for one of the most important theories on the role of the hippocampus. The rat uses spatial information provided by the distal cues in the room to efficiently locate the baited arms. The radial arm-maze allows the study of spatial reference and working memory processes in the rat. In reference memory procedures, information is useful for many sessions/days and may usually be needed during the entire experiment. On the contrary, working memory procedures have a major temporal component as the information presented in the maze is useful for one session but not for subsequent ones; the rat has to remember the information during a delay interval. Correct choices in the radial arm-maze are rewarded by food.

Procedure: The apparatus is a wooden elevated eight-arm radial maze with the arms extending from a central platform 26 cm in diameter. Each arm is 56 cm long and 5 cm wide with 2 cm high rails along the length of the arm. The maze is well illuminated and numerous cues are present. Food pellets (reward) are placed at the end of the arms. During the test, rats are fed once a day and their body weights maintained at 85% of their free feeding weight to motivate the rat to run the maze. Animals are trained on a daily basis in the maze to collect the food pellets. The session is terminated after 8 choices and the rat has to obtain the maximum number of rewards with a minimum number of errors.

Evaluation: The number of errors is counted during the session.

4. **Visual discrimination**

Principle: Vision is better than any other sensory system for the analysis of spatial relationships in the environment of the animal. From the retina to the cerebral cortex, the organization of the visual system ensures processing of visual information according to simple principles i.e. by fitting the distribution of light over the receptive surface to elementary geometrical concepts and by comparing these patterns with images stored in the memory.

Procedure: Rats and mice of both sexes are used and maintained under standard conditions. The apparatus consists of a square 10x10 cm start area separated by a Plexiglas sliding door from the choice area, which is connected by swing doors to the goal compartment. The grid floor in the starting and the choice areas is electrifiable. The stimulus can be attached to the swing doors. The patterns are black on a white

background and have different forms. The apparatus is illuminated by a dim light. The animal is placed into the apparatus with all doors open and allowed to explore it. Then it is placed in the start and after 5 s released by raising the Plexiglas door. After another 5 s, electric shocks (1 mA, 50 Hz, 0.5 s, 1/3 s) are applied until the animal escapes through either of the open doors to the safe goal compartment where it is left for some seconds. As soon as this preliminary step is mastered, the stimulus cards are inserted, the negative door is locked and the grid section in front of this door is electrified. The animal is trained to a criterion. On the next day the animal is retrained to the same criterion and retention is expressed in savings. Another parameter which can be used to evaluate the savings is the cumulative number of errors until the criterion is reached.

Evaluation: The number of correct answers as well as the number of trials until the criterion is reached is counted.

5. **Spatial learning in the water maze**

Principle: A task was developed where rats learn to swim in a water tank to find an escape platform hidden under the water. As there are no proximal cues to mark the position of the platform, the ability to locate it efficiently will depend on the use of a configuration of the cues outside the tank. Learning is reflected on the shorter latencies to escape and the decrease on the length of the path to find the platform. Although rodents can find the platform by using non-spatial strategies, the use of a spatial strategy is the most efficient way to escape and young animals develop the spatial strategy after a small number of trials.

Procedure: Different strains of rats are generally used. The apparatus is a circular water tank filled to a depth of 20 cm with 25 °C water. Four points equally distributed along the perimeter of the tank serve as starting locations. The tank is divided in four equal quadrants and a small platform (19 cm height) is located in the centre of one of the quadrants. The platform remains in the same position during the training days. The rat is released into the water and allowed 60–90 s. to find the platform. Animals usually receive 2–4 trials per day for 4–5 days until they escape onto the platform. Well trained rats escape in less than 10 s.

Evaluation: The latency to reach the escape platform is measured during the training days. A free-swim trial is generally performed after the training days where the escape platform is removed and the animal is allowed to swim for 30 s. With the help of a video system, the latency to reach the previous position of the platform, the number of annulus crossings as well as the time the rat spent in the training quadrant is measured. Well-trained rats show short latencies, a large number of annulus crossings and bias to the quadrant where the escape platform was located during the training sessions.

6. **Olfactory learning**

Principle: Odors provide rodents with important information on the environment and the learning of successive olfactory discrimination problems in rats is closely related to the acquisition rules of higher primates. Odor-reward associations are learned in few trials as odors exert more discriminative control over other sensory modalities like tones or lights. Animals have to learn to discriminate an arbitrary designated positive odor (i.e., banana) from a negative one (i.e., orange) to receive a reward.

Procedure: Rats are generally used. Animals are deprived of water for 48 h before the training and during the test they receive ad libitum water for only 30 min. The olfactory apparatus is a rectangular box (30 x 30 x 55 cm) with a photosensitive cell mounted on top of the water spout/odor outlet. Rats are trained to approach the water spout and to break the light beam. Responses to the positive odor are rewarded with water while responses to the negative odor results in the presentation of a light flash. The inter trial interval before the presentation of a new odor is usually 15 s. and the sessions last 30 min per day. Sessions are terminated when the rat makes 90% correct choices or after 400 trials.

Evaluation: The animal is rewarded with 0.05 ml of water when it breakes the beam to the positive odor or when it does not respond to the negative odor. Results are reported as the % correct responses or as a logit transformation of the % correct/incorrect response ratio.

7. **Hole board discrimination learning**

Principle: Here, we aimed at developing a higher throughput mouse spatial learning task using a holeboard chamber, an apparatus that has primarily been used to look at exploratory behaviors, not complex spatial learning. Holeboard-learning paradigms for rats have been described. A few studies also showed that mice could learn to retrieve a food pellet located in one hole out of four in a holeboard task setup. However, the simplicity of the task used in these mouse studies precluded a direct assessment of reference and working memory performances. Thus, as a starting point, we chose to adapt to mice a protocol similar to that described by vander Stay et al. (1990).

The holeboard apparatus consists of an open-field chamber with a 16-hole floor insert. Across trials, animals have to learn that the same four holes of 16 are always baited. After habituation to the apparatus, mice undergo four to six trials day across multiple days. Moreover, daily pretreatment with 0.1 and 1 mg/kg of scopolamine induces significant learning impairment compared with vehicle or 0.01 mg/kg. Thus, the present results suggest that this learning task could be used as an alternative approach to assess spatial discrimination performance in mice and may be useful for future pharmacological or behavioral phenotyping studies.

Apparatus: The holeboard discrimination task was conducted in test chambers corresponding to the rat open-field activity system provided by MED Associates Inc. (St. Albans, VT) and housed in sound-attenuated cubicles. The large test chambers (44.5x44.5 x30.5 cm) were originally developed for rats and were modified by the manufacturer with our input to allow for monitoring of mice. IR beam intensity was increased to reduce the spread of the beam, which increased activity monitoring precision. Two arrays of IR beams were used to track activity on the floor and nosepokes into the holes. The beams were adjusted so that the mice had to dip their head 8–9 mm to break an IR beam. A nosepoke was recorded when the IR beams were broken for three consecutive 50-ms samplings. The holeboard chambers contained a 16-hole holeboard floor insert. This three-piece assembly was placed in the bottom of the holeboard chamber. A specially made stainless steel floor with 0.95-cm legs was then placed on top of the three-piece assembly in the holeboard chamber. The stainless steel floor has four rows of four holes that are equidistant apart. The holes are 1.5 cm in diameter and spaced 6 cm apart. To preclude animals to use olfactory clues, all 16 holes were filled with 45-mg food pellets that were inaccessible and that were changed daily.

Procedure: In this test, an animal's ability to remember which four of 16 equidistant holes are baited with food was measured. On the first day, food-restricted mice received a single 15-min habituation session to acclimate to the apparatus. During this session, mice had to collect all the food pellets that were placed in every hole. The MED Associates ACTIVITY MONITOR software (version 4.0) automatically ended the session after all the pellets had been collected or after 15 min had elapsed, whichever came first. The training period began the following day; depending on the experiment, mice were trained for at least 4 consecutive days. Each daily session consisted of four or six 3-min trials with an intertrial interval of 3 min. The start position of a mouse was randomly changed across trials. The same four holes were baited with a 20-mg food pellet during each trial. The software automatically ended a trial when all four pellets had been collected or after 3 min had elapsed. After each trial, mice were returned to the home cage, and the holeboard floor insert was cleaned with alcohol to homogenize potential olfactory traces.

Evaluation: The average number of errors per trial an animal made each day was used as a measure of cognitive performance. Errors consisted of entering a hole that was never baited, re-entering a hole (working-memory error) or missing a baited hole (error of omission). When appropriate, we also analyzed the task completion time and the number of head entries. Learning curves were analyzed utilizing repeated-measures ANOVAs. Also, one-way ANOVAs followed by posthoc analyses (Student Newman–Keuls test) were used to compare overall performances between groups. Statistical analyses

were performed utilizing the STATVIEW5 software package.

Conditioned nictitating membrane response in rabbits

Principle: The rabbit's classically conditioned eye blink response has become a widely used model system for studying associative learning in mammals and to find drugs potentially useful in the treatment of age-related memory disorders.

Procedure: A small loop of surgical nylon is sutured into the right nictitating membrane, and the surrounding hair is removed. One day later, the rabbit is placed in a Plexiglas restrainer, and two stainlesssteel wound clips are applied to the skin over the parietal region. The rabbit is fit with a headmount that supports a photoresistive assembly for recording the nictitating membrane response by physical coupling with a length of thread to the nylon loop in the nictitating membrane. The transducer assembly converts nictitating membrane movements into electrical signals that are subjected to an analogto digital conversion using a 5-ms sampling rate and a resolution of 0.06 mm actual membrane extension. The animal is then positioned in a ventilated, soundattenuated chamber facing a stimulus panel containing an 11.4 -cm speaker and two 6-W, 24-V DC house lights, one mounted at each side of the speaker. During the course of the experiment, two stimuli are employed as conditioned stimulus: a) a 1000-ms, 1-kHz, 84-dB tone; b) a 1000-ms, intermittently presented light produced by interruption of the house lights at 10 Hz to yield a change in illumination, measured at the eye level of the rabbit from 32.11 to 8.01. The unconditioned stimulus is a 100-ms, 3-mA, 60Hz shock delivered to the wound clips by a constant current shock generator. Drug solutions or saline are injected subcutaneously into the cervical area of the rabbit via an infusion pump at a rate of 3 ml/min, 30 min before behavioral testing. Experimentally naive rabbits are randomly assigned in equal numbers to each of the treatments (n = 10 per treatment). The experiment consists of two phases: Phase 1 is an adaptation day followed by 9 days of acquisition training. No stimuli are presented during the 60-min adaptation session. Subjects are injected with their assigned treatment 30 min before each acquisition session. Each acquisition session consists of 30 tone-shock and 30 light-shock trials presented in a randomized sequence within 10 trial blocks, with the restriction that no more than three consecutive tone or light trials can occur. On each conditioned stimulus unconditioned stimulus trial, the offset of the 1 000- ms tone or light conditioned stimulus occurs simultaneously with the onset of the 100-ms unconditioned stimulus. The inter-trial interval is about 60 s. A response is defined as at least a 0.5-mm extension of the nictitating membrane. Responses occurring during the tone or light conditioned stimulus, but before the unconditioned stimulus

are recorded as conditioned response; those occurring after the unconditioned stimulus onset are recorded as unconditioned response.

Evaluation: The data are analyzed by repeated measures analyses of variance and Tukey tests. The significance level is set at $p < 0.05$.

Transgenic animal model

Principle: Mutation responsible for the rare cases of familial Alzheimer disease (FAD)—only to realize that not one but numerous mutations were actually able to cause the disease. Mutations are indeed located not only in the APP gene from which the Aβ peptide is cleaved, but also in the genes of presenilin 1 or 2 that are directly involved in Aβ production from APP. All of the mutations that have been tested; when transfected in cellular models, induce an increase in the Aβ42/Aβ40 ratio with the noticeable exception of the Arctic mutation directly involving the A_ sequence itself. All mutations induce an overproduction of Aβ except the mutation V715M, in which, however, the ratio Aβ42/ Aβ40 is increased. Transfecting the APP gene induces an overproduction of the protein, and APP overproduction may be sufficient to increase Aβ peptide secretion: both the β and the γ enzymatic activities do not appear to be rate limiting. There are two transgenic models:

- Amyloid-β Transgenic Mouse Models
- Tau Transgenic Mouse Models

Procedure: Twenty-seven APP-PS1 (15 males and 12 females) mice and 30 female B6_SJL wild-type mice spanning in age from 66 to 904 days were used in this study. Some of the mice were scanned repeatedly. Eight mice were scanned twice, and two were scanned three times. Additionally, three 624-day-old APP mice were scanned. Mice were anesthetized by using 1.0–1.5% isoflurane and O2/NO2 and positioned in a custom built device to immobilize the head during experiments. Body temperature was maintained at 37°C by warm water circulation, and physiological monitoring was used for temperature, respiration, and ECG.

ANANGESIC, ANTIPYRETIC AND ANTIINFLAMMATORY

ANALGESIC MODELS

Pain is a symptom of many diseases requiring treat ment with analgesics. Severe pain due to cancer metas tases needs the use of strong analgesics, that means opioid drugs. The addiction liability of opioids led to intensive research for compounds without this side ef fect. Many approaches have been used to

differentiate the various actions of strong analgesics by developing animal models not only for analgesic activity but also for addiction liability. Several types of opioid receptors have been identified in the brain allowing in vitro bind ing tests. However, the in vitro tests can only partially substitute for animal experiments involving pain. Pain is a common phenomenon in all animals, at least in vertebral animals, similar to that felt by man. Analgesic effects in animals are comparable with the thera peutic effects in man.

Animal models of nociception have two important components: the method of insult and the subsequent end-point measurement. The most appropriate models, whether an injury, application of chemical agents, or other manipulations, should produce nociception by recapitulating the mechanisms of specific clinical conditions. Similarly, measures of nociceptive behavior must not only detect pain-like responses, but should do so in a manner consistent with the clinical experience of pain. Measures of reflexive behaviors such as withdrawal thresholds to noxious stimuli have been used for decades to examine mechanisms of pain. These have clearly proven useful in advancing our understanding of the physiological basis of nociception, identification of neurotransmitters, receptors, intracellular messengers, and genes involved in pain behaviors; and better understanding of existing pharmacological and non-pharmacological pain treatments Further, over the last several decades, the pharmacological action (e.g. efficacy, potency, duration of action) of a broad spectrum of analgesics to reduce reflexive sensory responses in rodent models of acute nociception and chronic pain have demonstrated consistent correspondence to human analgesia

1. <u>In Vivo Animal Models</u>

These are **whole-animal experiments** used to study pain perception and analgesic activity. They are the **most commonly used models in pharmacological screening**.

A. Thermal (Heat-Induced) Pain Models

Used mainly for **centrally acting analgesics** such as Morphine.

Model	Animal Used	Principle
Hot Plate Test	Mouse/Rat	Animal placed on heated surface; latency to lick paw or jump is measured.
Tail Flick Test	Rat/Mouse	Tail exposed to radiant heat; withdrawal time indicates analgesic effect.

Model	Animal Used	Principle
Tail Immersion Test	Rat/Mouse	Tail dipped in hot water; increased withdrawal time indicates analgesia.

B. Chemical-Induced Pain Models

Used to test **peripheral analgesics** like Aspirin and Diclofenac.

Model	Pain Inducer	Principle
Acetic Acid Writhing Test	Acetic acid	Causes abdominal constrictions (writhing); reduction indicates analgesia.
Formalin Test	Formalin injection in paw	Produces biphasic pain (early neurogenic & late inflammatory phase).
Capsaicin-Induced Pain Test	Capsaicin	Activates pain receptors causing licking/biting behavior.

C. Mechanical Pain Models

Model	Principle
Von Frey Filament Test	Mechanical pressure applied to paw to measure withdrawal threshold.
Randall–Selitto Test	Increasing pressure applied to paw until withdrawal occurs.

D. Inflammatory Pain Models

Model	Principle
Carrageenan-Induced Paw Edema	Injection of carrageenan produces inflammation and pain.
Complete Freund's Adjuvant (CFA) Model	Chronic inflammation causing persistent pain.

E. Neuropathic Pain Models

Model	Principle
Chronic Constriction Injury (CCI)	Ligating sciatic nerve to induce neuropathic pain.
Spared Nerve Injury (SNI)	Partial nerve damage causing hypersensitivity.
Partial Sciatic Nerve Ligation (PSNL)	Produces neuropathic pain similar to human neuropathy.

2. In Vitro Models

These models use **cells, tissues, or receptors** to study analgesic mechanisms.

Model	Principle
Cyclooxygenase (COX) Enzyme Inhibition Assay	Measures inhibition of COX enzymes involved in prostaglandin synthesis.
Opioid Receptor Binding Assay	Determines binding affinity to opioid receptors.
Neuronal Cell Culture Pain Models	Study neurotransmitter release involved in pain pathways.

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3. Ex Vivo Models

These involve **isolated tissues taken from animals**.

Model	Principle
Isolated Nerve Preparation	Measures nerve conduction and pain signal transmission.
Spinal Cord Slice Models	Study synaptic transmission in pain pathways.

4. In Silico / Computational Models

Modern drug discovery approaches.

Model	Principle
Molecular Docking	Predicts binding of analgesic drugs to receptors.
QSAR Models	Correlates chemical structure with analgesic activity.

1. Hot Plate Test

Principle

The hot plate test is based on the response of animals to **thermal stimulus**. When placed on a heated surface, animals show pain responses such as paw licking or jumping. Centrally acting analgesics increase the **pain threshold** and prolong reaction time.

Procedure

1. Albino mice or rats are used and divided into control, standard, and test groups.
2. The hot plate apparatus is maintained at **55 ± 0.5°C**.
3. Each animal is placed individually on the hot plate.
4. The **latency time** for pain response (paw licking or jumping) is recorded.
5. Test drug and standard drug are administered.
6. Reaction time is measured again at intervals such as **30, 60, and 90 minutes**.

7. A **cut-off time (15 seconds)** is maintained to prevent tissue damage.

Evaluation

- Analgesic activity is indicated by **increase in reaction time** compared to control.
- Percentage increase in latency is calculated.

2. Tail Flick Test

Principle
The tail flick test measures the spinal reflex response to a **radiant heat stimulus** applied to the tail. Centrally acting analgesics increase the **tail withdrawal latency**.

Procedure

1. Rats or mice are gently restrained.
2. A beam of radiant heat is focused on the middle portion of the tail.
3. Time taken for the animal to flick its tail is recorded.
4. Test drug or standard drug is administered.
5. Tail flick latency is measured at different time intervals.

Evaluation

- Increased **tail flick latency** indicates analgesic activity.
- A cut-off time (10–12 sec) is set to avoid tissue injury.

3. Tail Immersion Test

Principle
Pain is produced by immersing the tail of the animal in hot water. Analgesic drugs increase the time taken for tail withdrawal.

Procedure

1. The distal part of the rat's tail is immersed in water maintained at **55°C**.
2. The time taken for tail withdrawal is recorded.
3. Test drug or standard drug is administered.
4. Withdrawal latency is measured again at specific intervals.

Evaluation

- Increased **tail withdrawal time** indicates analgesic activity.

B. Chemical-Induced Pain Models

Used mainly to evaluate **peripheral analgesics** such as Aspirin and Diclofenac.

4. Acetic Acid–Induced Writhing Test

Principle
Intraperitoneal injection of acetic acid causes abdominal constrictions due to release of inflammatory mediators like prostaglandins. Peripheral analgesics reduce the number of writhes.

Procedure

1. Mice are divided into control, standard, and test groups.
2. Test drug or standard drug is administered.
3. After 30 minutes, **0.6% acetic acid** is injected intraperitoneally.
4. Animals show characteristic writhing movements such as abdominal contraction and hind limb extension.
5. The number of writhes is counted for **20 minutes**.

Evaluation

- Decrease in number of writhes indicates analgesic activity.

Formula:

% Inhibition=Control−TestControl×100\%\,Inhibition = \frac{Control - Test}{Control} \times 100%Inhibition=ControlControl−Test×100

5. Formalin Test

Principle
Injection of formalin into the paw produces **biphasic pain response**:

- Early phase (neurogenic pain)
- Late phase (inflammatory pain)

Procedure

1. Formalin (2–5%) is injected into the **hind paw of mice or rats**.
2. Animals show licking, biting, or shaking of the injected paw.
3. Test drug or standard drug is administered before formalin injection.
4. Time spent licking the paw is recorded.

Evaluation

- Pain response is measured in two phases:
  - **Early phase:** 0–5 minutes
  - **Late phase:** 15–30 minutes
- Reduction in licking time indicates analgesic activity.

6. Capsaicin-Induced Pain Test

Principle
Capsaicin stimulates nociceptive sensory neurons and produces intense pain responses such as licking or biting.

Procedure

1. Capsaicin solution is injected into the hind paw of mice.
2. Animals display licking or biting of the paw.
3. Test drug or standard drug is administered before capsaicin injection.
4. Pain behavior is observed for **5–10 minutes**.

Evaluation

- Decrease in paw licking or biting time indicates analgesic activity.

C. Mechanical Pain Models

7. Von Frey Filament Test

Principle
Mechanical stimuli applied to the paw produce withdrawal response. Analgesics increase the **mechanical threshold for pain**.

Procedure

1. Animals are placed on a wire mesh platform in individual cages.
2. Von Frey filaments of increasing force are applied to the plantar surface of the paw.
3. Each filament is applied until the animal withdraws the paw.

Evaluation

- Increased **withdrawal threshold** indicates analgesic activity.

8. Randall–Selitto Test

Principle
Pain is produced by applying increasing mechanical pressure to the paw. Analgesics increase tolerance to pressure.

Procedure

1. The rat paw is placed under the Randall–Selitto apparatus.
2. Pressure is gradually increased.
3. The point at which the animal withdraws the paw or vocalizes is recorded.

Evaluation

- Increased **pressure threshold** indicates analgesic activity.

D. Inflammatory Pain Models

9. Carrageenan-Induced Paw Edema

Principle
Injection of carrageenan produces inflammation and pain through release of inflammatory mediators like prostaglandins.

Procedure

1. Carrageenan solution is injected into the rat hind paw.
2. Test drug or standard drug is administered before or after injection.
3. Paw volume is measured using a plethysmometer at **1, 2, and 3 hours**.

Evaluation

- Decrease in paw edema indicates anti-inflammatory analgesic activity.

10. Complete Freund's Adjuvant (CFA) Model

Principle
Injection of CFA produces chronic inflammation and persistent pain.

Procedure

1. CFA is injected into the hind paw of rats.
2. Inflammation and hypersensitivity develop within 24 hours.
3. Mechanical and thermal pain responses are measured.

Evaluation

- Reduction in hyperalgesia or paw swelling indicates analgesic effect.

E. Neuropathic Pain Models

11. Chronic Constriction Injury (CCI)

Principle
Loose ligation of the sciatic nerve causes neuropathic pain due to nerve injury.

Procedure

1. Sciatic nerve of rat is exposed surgically.
2. Several loose ligatures are tied around the nerve.
3. Animals develop hyperalgesia and allodynia.

Evaluation

- Pain sensitivity measured using thermal or mechanical tests.

12. Spared Nerve Injury (SNI)

Principle
Selective injury to certain branches of the sciatic nerve produces neuropathic pain.

Procedure

1. Tibial and common peroneal nerves are ligated and cut.
2. The sural nerve is left intact.
3. Hypersensitivity develops after surgery.

Evaluation

- Mechanical hypersensitivity measured using Von Frey filaments.

13. Partial Sciatic Nerve Ligation (PSNL)

Principle
Partial ligation of the sciatic nerve induces neuropathic pain similar to human neuropathy.

Procedure

1. Sciatic nerve is partially ligated with a suture.
2. Animals develop mechanical and thermal hypersensitivity.

Evaluation

- Reduction in hyperalgesia indicates analgesic activity.

. In Vitro Models

14. Cyclooxygenase (COX) Enzyme Inhibition Assay

Principle
Measures inhibition of COX enzymes responsible for prostaglandin synthesis.

Procedure

1. COX enzyme is incubated with arachidonic acid substrate.
2. Test compound is added.
3. Prostaglandin production is measured.

Evaluation

- Decrease in prostaglandin production indicates analgesic potential.

15. Opioid Receptor Binding Assay

Principle
Determines the binding affinity of compounds to opioid receptors.

Procedure

1. Membrane preparations containing opioid receptors are used.
2. Radiolabeled ligand is added with test compound.
3. Binding displacement is measured.

Evaluation

- Higher receptor binding affinity indicates potential analgesic activity.

16. Neuronal Cell Culture Pain Models

Principle
Used to study neurotransmitter release and signaling involved in pain.

Procedure

1. Neuronal cells are cultured in vitro.
2. Pain mediators are applied.
3. Test drugs are added.

Evaluation

- Changes in neurotransmitter release are measured.

Ex Vivo Models

17. Isolated Nerve Preparation

Principle
Isolated nerve tissues are used to study conduction of pain signals.

Procedure

1. Nerve tissue is isolated from experimental animals.
2. Electrical stimuli are applied.
3. Drug effects on nerve conduction are recorded.

Evaluation

- Reduction in nerve conduction indicates analgesic effect.

18. Spinal Cord Slice Model

Principle
Used to study synaptic transmission in spinal cord pain pathways.

Procedure

1. Spinal cord slices are prepared from animals.
2. Electrical stimulation is applied.
3. Drug effects on neurotransmission are observed.

Evaluation

- Decrease in synaptic transmission indicates analgesic activity.

In Silico / Computational Models

19. Molecular Docking

Principle
Predicts binding of analgesic compounds with target receptors.

Procedure

1. 3D structure of receptor is obtained.
2. Test compound structure is docked computationally.
3. Binding interactions are analyzed.

Evaluation

- Strong binding affinity indicates potential analgesic activity.

20. QSAR (Quantitative Structure–Activity Relationship)

Principle
Correlates chemical structure with biological activity.

Procedure

1. Chemical descriptors of compounds are calculated.
2. Statistical models are developed.
3. Biological activity is predicted.

Evaluation

- Compounds with favorable QSAR values are selected as analgesic candidates.

ANTIPYRETIC MODELS

Fever (pyrexia) is a rise in body temperature above the normal range due to infection, inflammation, or immune reactions. It occurs when pyrogenic substances stimulate the thermoregulatory center in the hypothalamus, leading to increased production of mediators such as Prostaglandin E2. These mediators elevate the hypothalamic set point, resulting in an increase in body temperature.

Antipyretic drugs reduce fever by inhibiting the synthesis of prostaglandins in the hypothalamus. Common standard antipyretic drugs include Paracetamol and Aspirin. These drugs act mainly through inhibition of the cyclooxygenase (COX) enzyme involved in prostaglandin synthesis.

According to the pharmacological assays described in the Vogel book, antipyretic activity is commonly evaluated using **experimental animal models in which fever is artificially induced**. The most widely used model is the **brewer's yeast–induced pyrexia model**, which mimics fever conditions seen during infections. In this method, injection of yeast produces fever through the release of endogenous pyrogens and increased prostaglandin production.

Other experimental models described include **lipopolysaccharide-induced fever, turpentine-induced pyrexia, and prostaglandin-induced fever models**. These models help researchers study the mechanism of fever and evaluate the effectiveness of new antipyretic compounds.

The Vogel methods provide **standardized experimental procedures**, ensuring reliable evaluation of antipyretic activity in preclinical studies. These assays are widely used in pharmacological research and drug development to screen potential antipyretic agents before clinical trials.

Category	Models
In Vivo Models	Yeast-induced pyrexia, LPS-induced fever, Turpentine-induced fever, Amphetamine-induced hyperthermia, Prostaglandin-induced fever, Milk-induced pyrexia
In Vitro Models	COX inhibition assay, Cytokine release assay, Prostaglandin production assay
Ex Vivo Models	Hypothalamic tissue studies, Macrophage culture

Category	Models
In Silico Models	Molecular docking, QSAR analysis

. **In Vivo Models**

1. Yeast-Induced Pyrexia Model

Principle

Subcutaneous injection of brewer's yeast induces fever by increasing the production of endogenous pyrogens and prostaglandins, especially Prostaglandin E2 in the hypothalamus. Antipyretic drugs reduce the elevated body temperature.

Procedure

1. Healthy rats or mice are selected and their normal rectal temperature is recorded.
2. Fever is induced by subcutaneous injection of **15–20% brewer's yeast suspension**.
3. Animals are kept fasting for 18–24 hours to allow fever development.
4. Rectal temperature is measured again to confirm pyrexia.
5. Test drug or standard drug is administered orally or intraperitoneally.
6. Rectal temperature is recorded at **1, 2, 3, and 4 hours** after drug administration.

Evaluation

- Reduction in rectal temperature compared with control indicates antipyretic activity.

2. Lipopolysaccharide (LPS)-Induced Fever Model

Principle

Lipopolysaccharide from Gram-negative bacteria induces fever by stimulating release of cytokines such as IL-1 and TNF which increase prostaglandin production in the hypothalamus.

Procedure

1. Baseline body temperature of animals is recorded.
2. LPS is injected intraperitoneally to induce fever.
3. Body temperature increases within a few hours.
4. Test drug or standard drug is administered.
5. Temperature is measured at regular intervals.

Evaluation

- Decrease in elevated body temperature indicates antipyretic activity.

3. Turpentine Oil–Induced Pyrexia

Principle
Injection of turpentine oil produces inflammation and release of endogenous pyrogens, leading to fever.

Procedure

1. Baseline rectal temperature of animals is recorded.
2. Turpentine oil is injected subcutaneously.
3. Fever develops within a few hours.
4. Test drug or standard antipyretic is administered.
5. Body temperature is monitored periodically.

Evaluation

- Reduction in fever compared to control animals indicates antipyretic effect.

4. Amphetamine-Induced Hyperthermia

Principle
Amphetamine increases metabolic activity and stimulates the central nervous system, leading to hyperthermia.

Procedure

1. Baseline body temperature is recorded.
2. Amphetamine is administered to animals.
3. Hyperthermia develops due to increased metabolic rate.
4. Test drug or standard antipyretic is administered.
5. Temperature is measured at intervals.

Evaluation

- Reduction in elevated temperature indicates antipyretic activity.

5. Prostaglandin-Induced Fever Model

Principle
Injection of prostaglandins such as Prostaglandin E2 into the hypothalamus causes fever by increasing the thermoregulatory set point.

Procedure

1. Animals are anesthetized and prostaglandin solution is injected into the hypothalamic region.
2. Fever develops rapidly.
3. Test drug or standard drug is administered.
4. Body temperature is monitored.

Evaluation

- Reduction in temperature indicates inhibition of prostaglandin-mediated fever.

6. Milk-Induced Pyrexia

Principle
Injection of milk proteins causes immune reactions and release of endogenous pyrogens, resulting in fever.

Procedure

1. Baseline body temperature of animals is recorded.
2. Milk suspension is injected subcutaneously.
3. Fever develops within a few hours.
4. Test drug or standard drug is administered.
5. Temperature is recorded at regular intervals.

Evaluation

- Decrease in body temperature indicates antipyretic effect.

In Vitro Models

7. Cyclooxygenase (COX) Inhibition Assay

Principle
Antipyretic drugs reduce fever by inhibiting cyclooxygenase enzymes responsible for prostaglandin synthesis.

Procedure

1. COX enzyme preparation is incubated with arachidonic acid substrate.
2. Test compound is added to the reaction mixture.
3. Prostaglandin formation is measured using biochemical assays.

Evaluation

- Reduced prostaglandin production indicates antipyretic potential.

8. Cytokine Release Assay

Principle
Fever is mediated by cytokines such as IL-1 and TNF released from immune cells.

Procedure

1. Immune cells such as macrophages are cultured in vitro.
2. Pyrogens like LPS are added to stimulate cytokine production.
3. Test compounds are added.
4. Cytokine levels are measured using ELISA or similar assays.

Evaluation

- Reduction in cytokine levels indicates potential antipyretic activity.

9. Prostaglandin Production Assay

Principle
Measures inhibition of prostaglandin production in cultured cells.

Procedure

1. Cells capable of producing prostaglandins are cultured.
2. Pyrogenic stimulus is added.
3. Test drug is introduced.
4. Prostaglandin levels are measured.

Evaluation

- Lower prostaglandin levels indicate antipyretic activity.

Ex Vivo Models

10. Hypothalamic Tissue Studies

Principle
The hypothalamus controls body temperature. Drugs affecting prostaglandin production in hypothalamic tissues may show antipyretic effects.

Procedure

1. Hypothalamic tissues are isolated from experimental animals.
2. Tissues are incubated with pyrogenic substances.
3. Test compounds are added.
4. Prostaglandin production is measured.

Evaluation

- Reduced prostaglandin synthesis indicates antipyretic potential.

11. Macrophage Culture

Principle
Macrophages release pyrogenic cytokines responsible for fever.

Procedure

1. Macrophages are isolated from animal tissues.
2. Cells are cultured in laboratory conditions.
3. Pyrogenic stimuli are added.
4. Test drugs are introduced.

Evaluation

- Reduction in cytokine release indicates antipyretic activity.

In Silico Models
12. Molecular Docking

Principle
Predicts interaction of drug molecules with target proteins involved in fever pathways such as COX enzymes.

Procedure

1. 3D structure of the target protein is obtained.

2. Test compound structure is introduced into docking software.
3. Binding interactions between ligand and receptor are analyzed.

Evaluation

- Strong binding affinity suggests potential antipyretic activity.

13.QSAR (Quantitative Structure–Activity Relationship)

Principle
Correlates chemical structure with biological activity to predict antipyretic effects.

Procedure

1. Chemical descriptors of compounds are calculated.
2. Statistical models are developed linking structure and activity.
3. New compounds are analyzed using the model.

Evaluation

- Compounds with favorable QSAR parameters are predicted to have antipyretic activity.

ANTIINFLAMMATORY MODELS

Inflammation is a **protective biological response of the body to tissue injury, infection, or irritation**. It involves a complex interaction of **vascular, cellular, and chemical mediators** that help eliminate harmful stimuli and initiate tissue repair. However, excessive or prolonged inflammation can lead to **tissue damage and chronic diseases**, therefore the development of effective anti-inflammatory drugs is important in pharmacotherapy.

Inflammatory reactions are generally characterized by the **classical signs**: redness (*rubor*), swelling (*tumor*), heat (*calor*), pain (*dolor*), and sometimes loss of function. These responses occur due to the release of inflammatory mediators such as **histamine, serotonin, prostaglandins, leukotrienes, cytokines, and bradykinin**. Since different mediators participate in different phases of inflammation, various experimental models are required to study and evaluate the activity of anti-inflammatory drugs.

Classification of Anti-Inflammatory Models

Category	Models / Examples
1. Acute Inflammation Models	Carrageenan-induced rat paw edema, Histamine-induced paw edema, Serotonin-induced paw edema, Dextran-induced paw edema, Arachidonic acid-induced ear edema, Xylene-induced ear edema
2. Sub-Acute Inflammation Models	Cotton pellet-induced granuloma, Air pouch granuloma, Sponge implantation method
3. Chronic Inflammation Models	Freund's complete adjuvant-induced arthritis, Collagen-induced arthritis, Adjuvant-induced polyarthritis
4. Topical Inflammation Models	Croton oil-induced ear edema, Oxazolone-induced dermatitis, TPA-induced ear edema
5. In Vitro / Biochemical Models	Cyclooxygenase (COX-1 and COX-2) inhibition assay, Lipoxygenase inhibition assay, Prostaglandin synthesis inhibition
6. Cellular / Immunological Models	Leukocyte migration assay, Cytokine release assay, Macrophage activation assays

1. Carrageenan-Induced Rat Paw Edema (Acute Inflammation)

Principle
Carrageenan produces acute inflammation in the rat paw through the release of mediators such as **histamine, serotonin, bradykinin, and prostaglandins**. Anti-inflammatory drugs reduce the swelling caused by these mediators.

Procedure

- Rats are divided into control and test groups.
- Test drug is administered orally or intraperitoneally.
- After 1 hour, **0.1 ml of 1% carrageenan** is injected into the subplantar region of the rat hind paw.
- Paw volume is measured using a **plethysmometer** at intervals (0, 1, 2, 3, and 4 hours).

Evaluation
Anti-inflammatory activity is expressed as **percentage inhibition of paw edema** compared with the control group.

2. Histamine-Induced Paw Edema

Principle
Histamine is an early mediator of inflammation. Injection into the paw produces edema due to **vasodilation and increased vascular permeability**. Drugs that inhibit histamine-mediated inflammation reduce paw swelling.

Procedure

- Experimental animals are divided into groups.
- Test drug is administered before induction.
- **Histamine solution** is injected into the rat paw.
- Paw volume is measured at different time intervals.

Evaluation
Reduction in paw swelling indicates **antihistaminic or anti-inflammatory activity**.

3. Serotonin-Induced Paw Edema

Principle
Serotonin (5-HT) is another inflammatory mediator responsible for vascular permeability and edema formation.

Procedure

- Animals receive the test compound before induction.
- **Serotonin solution** is injected into the paw.
- Paw edema is measured with a plethysmometer.

Evaluation
The **percentage inhibition of paw edema** compared with the control group indicates anti-inflammatory activity.

4. Dextran-Induced Paw Edema

Principle
Dextran produces edema mainly by **mast cell degranulation and release of histamine and serotonin**.

Procedure

- Rats receive the test drug.
- **Dextran solution** is injected into the paw.
- Paw volume is measured periodically.

Evaluation
The **reduction in paw edema** compared with the control indicates inhibition of inflammatory mediators.

5. Arachidonic Acid-Induced Ear Edema

Principle
Arachidonic acid induces inflammation by producing **prostaglandins and leukotrienes** through the cyclooxygenase and lipoxygenase pathways.

Procedure

- Test drug is applied topically or administered systemically.
- **Arachidonic acid** is applied to the ear of mice.
- Ear thickness or weight is measured after a specific time.

Evaluation
Decrease in ear swelling compared with control indicates **inhibition of eicosanoid synthesis**.

6. Xylene-Induced Ear Edema

Principle
Xylene causes irritation and inflammation leading to **vasodilation and increased vascular permeability** in the ear.

Procedure

- Test compound is administered to mice.
- **Xylene** is applied to the ear surface.
- After a fixed time, ear discs are removed and weighed.

Evaluation
The **difference in ear weight** between treated and control animals indicates anti-inflammatory activity.

Sub-Acute Inflammation Models
7. Cotton Pellet-Induced Granuloma

Principle
Implanted cotton pellets stimulate **granuloma formation and fibroblast proliferation**, representing sub-acute inflammation.

Procedure

- Sterile cotton pellets are implanted subcutaneously in rats.
- Test drug is administered for several days.
- After 7 days, pellets are removed, dried, and weighed.

Evaluation
The **dry weight of granuloma tissue** indicates inflammatory response. Reduction suggests anti-inflammatory activity.

8. Air Pouch Granuloma

Principle
An air pouch created under the skin acts as a cavity where inflammatory exudate accumulates after irritant injection.

Procedure

- Air is injected subcutaneously to create a pouch.
- Irritant solution is injected into the pouch.
- Test drug is administered.
- Exudate volume and cell infiltration are measured.

Evaluation
Reduction in **exudate volume and leukocyte count** indicates anti-inflammatory effect.

9. Sponge Implantation Method

Principle
Implanted sponge acts as a matrix for **cell infiltration and connective tissue growth** during inflammation.

Procedure

- Sterile sponge pieces are implanted subcutaneously in animals.
- Test drug is administered daily.
- Sponges are removed after several days.

Evaluation
The **weight and histological examination of tissue growth** around the sponge indicate inflammatory response.

Chronic Inflammation Models
10. Freund's Complete Adjuvant-Induced Arthritis

Principle
Injection of **Freund's complete adjuvant (FCA)** produces chronic arthritis similar to **rheumatoid arthritis**.

Procedure

- FCA is injected into the rat paw.
- Test drugs are administered daily.
- Paw swelling and joint inflammation are monitored over several weeks.

Evaluation
Reduction in **paw swelling, arthritis score, and joint damage** indicates anti-arthritic activity.

11. Collagen-Induced Arthritis

Principle
Injection of **type II collagen** induces autoimmune arthritis similar to human rheumatoid arthritis.

Procedure

- Collagen is injected with an adjuvant.
- Test drugs are administered during disease progression.
- Joint inflammation and swelling are recorded.

Evaluation
Decrease in **arthritis severity and joint destruction** indicates anti-inflammatory or immunomodulatory activity.

12. Adjuvant-Induced Polyarthritis

Principle
Adjuvant injection produces **systemic polyarthritis**, affecting multiple joints.

Procedure

- Adjuvant is injected into the hind paw.
- Animals receive test drugs during the disease period.
- Swelling and joint involvement are observed.

Evaluation
The **reduction in arthritis index** indicates therapeutic activity.

In Vitro / Biochemical Models

13. Cyclooxygenase (COX-1 and COX-2) Inhibition Assay

Principle
Anti-inflammatory drugs inhibit **COX enzymes**, preventing prostaglandin formation.

Procedure

- Enzyme preparation is incubated with arachidonic acid.
- Test compounds are added.
- Prostaglandin formation is measured.

Evaluation
The **percentage inhibition of COX activity** indicates drug potency.

14. Lipoxygenase Inhibition Assay

Principle
Lipoxygenase converts arachidonic acid to **leukotrienes**, which are inflammatory mediators.

Procedure

- Enzyme solution is incubated with substrate and test drug.
- Product formation is measured spectrophotometrically.

Evaluation
Reduction in leukotriene formation indicates anti-inflammatory activity.

15. Prostaglandin Synthesis Inhibition

Principle
Measures the ability of compounds to inhibit **prostaglandin production** from arachidonic acid.

Procedure

- Enzyme or tissue preparations are incubated with test drugs.
- Prostaglandin levels are determined using biochemical assays.

Evaluation
Lower prostaglandin levels indicate **anti-inflammatory potential**.

Cellular / Immunological Models

16. Leukocyte Migration Assay

Principle
Inflammation involves migration of leukocytes to the site of injury.

Procedure

- Inflammation is induced in animals.
- Leukocyte movement into exudate or tissue is measured.

Evaluation
Reduced leukocyte migration indicates anti-inflammatory activity.

17. Cytokine Release Assay

Principle
Inflammatory cells release cytokines such as **TNF-α, IL-1, and IL-6**.

Procedure

- Immune cells are stimulated in vitro.
- Test drugs are added.
- Cytokine levels are measured using ELISA.

Evaluation
Reduction in cytokine production indicates anti-inflammatory effect.

18. Macrophage Activation Assay

Principle
Activated macrophages produce inflammatory mediators such as **nitric oxide and cytokines**.

Procedure

- Macrophages are cultured and stimulated with endotoxins.
- Test compounds are added to the culture.

Evaluation
Decrease in mediator production reflects anti-inflammatory activity.

GENERAL ANESTHETETIC MODELS

General anaesthetics are drugs that produce a **reversible loss of consciousness and sensation throughout the entire body**, allowing surgical and diagnostic procedures to be performed without pain or distress to the patient.

General anaesthesia is characterized by a combination of **hypnosis (loss of consciousness), analgesia (pain relief), muscle relaxation, and suppression of reflex responses**. These effects result from **depression of the central nervous system (CNS)**, particularly in the brain and spinal cord. The exact mechanism of action of general anaesthetics is complex and involves interaction with neuronal membranes and modulation of neurotransmitter systems such as **GABA, glutamate, and ion channels**.

Experimental pharmacology plays an important role in the **evaluation and development of new anaesthetic agents**. Various **animal models and pharmacological assays** are used to assess the onset, depth, duration, and recovery from anaesthesia. These models often measure parameters such as **loss of righting reflex, sleeping time, reflex suppression, and respiratory or cardiovascular effects**.

Thus, the experimental models described in Vogel's pharmacological assays help researchers **screen and compare potential anaesthetic compounds**, determine their **potency and safety**, and understand their pharmacological actions before they are tested in clinical trials.

Category	Animal Models / Tests
1. Hypnosis and Sleep Induction Models	Barbiturate-induced sleeping time test (Hexobarbital or Pentobarbital sleeping time), Ethanol-induced sleeping time
2. Loss of Righting Reflex Models	Loss and recovery of righting reflex test in mice or rats
3. Inhalational Anaesthetic Models	Minimum Alveolar Concentration (MAC) determination, Induction and recovery time measurement
4. Reflex Suppression Models	Corneal reflex test, Pedal withdrawal reflex test, Tail pinch reflex
5. Neuromuscular and Muscle Relaxation Models	Rotarod performance test, Grip strength test
6. Behavioral and CNS Depression Models	Locomotor activity test, Open field test
7. Analgesia During Anaesthesia Models	Tail flick test under anesthesia, Hot plate test
8. Physiological Monitoring Models	Measurement of respiration, blood pressure, heart rate during anesthesia

1. Barbiturate-Induced Sleeping Time Test (Hexobarbital or Pentobarbital)

Principle:

This test is based on the ability of general anaesthetic or CNS-depressant drugs to enhance or prolong barbiturate-induced sleep**. Barbiturates such as hexobarbital or pentobarbital produce sleep by depressing the central nervous system. Drugs with anaesthetic or sedative properties prolong the duration of sleep.**

Procedure:

Experimental animals such as mice or rats are divided into control and test groups. The test compound is administered prior to the barbiturate injection. After a fixed time, hexobarbital or pentobarbital is injected intraperitoneally to induce sleep. The animals are then observed for the onset of sleep, which is usually determined by the **loss of righting reflex**.

Evaluation:

The duration between the **loss and recovery of the righting reflex** is recorded as the sleeping time. An increase in sleeping time compared with the control group indicates potentiation of anaesthetic or CNS-depressant activity.

2. Loss of Righting Reflex Test

Principle:

General anaesthetics depress the central nervous system and cause **loss of the animal's ability to correct its body position** when placed on its back. This loss of righting reflex is widely used as an indicator of hypnosis and depth of anaesthesia.

Procedure:

Animals such as mice or rats receive the test anaesthetic drug either by injection or inhalation. After administration, each animal is placed on its back. If the animal fails to return to its normal upright position within a few seconds, the righting reflex is considered lost.

Evaluation:

The **time required for the loss of the righting reflex (induction time)** and the **time until recovery of the reflex (duration of anaesthesia)** are recorded. Shorter induction time and longer duration indicate stronger anaesthetic activity.

3. Inhalational Anaesthetic Models (Minimum Alveolar Concentration)

Principle:

The potency of inhalational anaesthetic agents is measured using **Minimum Alveolar Concentration (MAC)**, which represents the concentration of anaesthetic vapor required to prevent movement in response to a painful stimulus in 50% of animals.

Procedure:

Animals are placed in a chamber where a controlled concentration of inhalational anaesthetic is administered. The concentration is gradually adjusted while applying a standard noxious stimulus such as tail clamp or paw pinch. The concentration that prevents movement in half of the animals is determined.

Evaluation:

The MAC value is calculated. **Lower MAC values indicate higher anaesthetic potency**, while higher values suggest weaker anaesthetic activity.

4. Reflex Suppression Tests (Corneal, Pedal Withdrawal, Tail Pinch)

Principle:

During deep anaesthesia, protective reflexes such as **corneal reflex, pedal withdrawal reflex, and tail pinch response** are suppressed. Monitoring these reflexes helps determine the **depth of anaesthesia**.

Procedure:

Animals are administered the test anaesthetic drug. After induction, different reflexes are tested. For example, the corneal reflex is checked by gently touching the cornea, while the pedal withdrawal reflex is assessed by pinching the paw.

Evaluation:

The disappearance and reappearance of these reflexes are recorded. The **absence of reflex responses indicates adequate depth of anaesthesia**, while their return indicates recovery.

5. Neuromuscular and Muscle Relaxation Models (Rotarod and Grip Strength Tests)

Principle:

Many general anaesthetics produce **muscle relaxation and impairment of motor coordination**. Tests such as rotarod performance and grip strength are used to evaluate neuromuscular effects.

Procedure:

Animals are trained to walk on a rotating rod or to grasp a bar. After administration of the anaesthetic drug, their ability to maintain balance on the rotating rod or their grip strength is tested.

Evaluation:

A **decrease in the time the animal remains on the rotarod** or a reduction in grip strength indicates neuromuscular depression caused by the anaesthetic drug.

6. Behavioral and CNS Depression Models (Locomotor Activity and Open Field Test)

Principle:

General anaesthetics and CNS depressants reduce **spontaneous locomotor activity and exploratory behavior** in animals.

Procedure:

Animals are placed in an open field apparatus or locomotor activity chamber after drug administration. Their movement, number of crossings, and exploratory behavior are recorded for a specific period.

Evaluation:

A **reduction in locomotor activity** compared with the control group indicates CNS depressant or sedative effects of the drug.

7. Analgesia During Anaesthesia (Tail Flick and Hot Plate Tests)

Principle:

General anaesthetics may produce **analgesic effects by increasing the pain threshold**. Thermal stimuli such as heat are used to evaluate pain perception.

Procedure:

Animals are placed on a hot plate or exposed to a radiant heat source directed at the tail. The time taken for the animal to withdraw the paw or flick the tail is recorded after administration of the anaesthetic.

Evaluation:

An **increase in reaction time** compared with the control group indicates analgesic or antinociceptive activity during anaesthesia.

8. Physiological Monitoring Models

Principle:

General anaesthetic drugs influence **vital physiological functions**, including respiration, heart rate, and blood pressure.

Procedure:

Animals receiving anaesthetic drugs are connected to monitoring instruments to record physiological parameters during the anaesthetic period.

Evaluation:

Changes in **respiratory rate, heart rate, and blood pressure** are analyzed to determine the safety profile and systemic effects of the anaesthetic agent.

SEDATIVE AND HYPNOTICS MODELS

Sedatives and hypnotics are central nervous system (CNS) depressant drugs that reduce excitement and induce calmness or sleep. These agents are widely used in the management of **anxiety, insomnia, and other neurological conditions** where reduction of CNS activity is required.

Sedative drugs primarily **decrease mental activity and produce a calming effect without necessarily causing sleep**, while hypnotic drugs produce **drowsiness and facilitate the onset and maintenance of sleep**. The difference between sedatives and hypnotics is mainly **dose dependent**, as many sedative drugs can produce hypnosis at higher doses.

The pharmacological action of sedative–hypnotic agents is mainly associated with **depression of the central nervous system**, particularly through the enhancement of inhibitory neurotransmission in the brain. Many commonly used drugs in this class act by **modulating the gamma-aminobutyric acid (GABA) receptor system**, which plays a key role in regulating neuronal excitability.

In experimental pharmacology and drug discovery, several **animal models and behavioral assays** are used to evaluate the sedative and hypnotic effects of new compounds. These models assess parameters such as **sleep induction, sleeping time, loss of righting reflex, locomotor activity, and motor coordination**. Such experimental methods help researchers determine the **efficacy, potency, and safety** of potential sedative–hypnotic agents before they proceed to clinical trials.

Classification of Animal Models for Sedatives and Hypnotics

Category	Models / Tests
1. Sleep Induction and Hypnotic Models	Barbiturate-induced sleeping time test (Pentobarbital or Hexobarbital sleeping time), Ethanol-induced sleeping time
2. Loss of Righting Reflex Models	Loss and recovery of righting reflex test
3. Locomotor Activity Models	Spontaneous locomotor activity test, Actophotometer test
4. Behavioral Sedation Models	Open field test, Hole board test
5. Motor Coordination Tests	Rotarod performance test
6. Muscle Relaxant and CNS	Grip strength test, Traction test

Category	Models / Tests
Depression Models	
7. Exploratory Behavior Models	Rearing behavior test, Head-dipping test
8. EEG and Neurophysiological Models	Electroencephalographic (EEG) sleep analysis

1. Barbiturate-Induced Sleeping Time Test (Pentobarbital or Hexobarbital)

Principle:
This test is based on the ability of sedative and hypnotic drugs to **enhance or prolong barbiturate-induced sleep**. Barbiturates produce sleep by depressing the central nervous system. Compounds possessing sedative or hypnotic properties either **reduce the onset time of sleep or prolong the duration of sleep**.

Procedure:
Experimental animals such as mice or rats are divided into control and test groups. The test compound is administered prior to the injection of pentobarbital or hexobarbital. After a fixed interval, the barbiturate is injected intraperitoneally to induce sleep. The animals are then observed for the **loss of righting reflex**, which indicates the onset of sleep.

Evaluation:
The time between **loss and recovery of the righting reflex** is recorded as the sleeping time. A **significant increase in sleeping time** compared with the control group indicates sedative-hypnotic activity.

2. Ethanol-Induced Sleeping Time

Principle:
Ethanol produces central nervous system depression and sleep at higher doses. Sedative and hypnotic drugs can **potentiate ethanol-induced sleep**.

Procedure:
Animals receive the test compound before ethanol administration. Ethanol is then injected intraperitoneally to induce sleep. The onset of sleep is determined by observing the loss of the righting reflex.

Evaluation:
The **duration of sleep and time to recovery of righting reflex** are recorded. Prolongation of sleeping time compared with control animals indicates sedative or hypnotic activity.

3. Loss of Righting Reflex Test

Principle:
Sedative and hypnotic drugs depress the central nervous system and may cause the animal to **lose its**

ability to regain an upright position when placed on its back. This loss of righting reflex is used as an indicator of hypnosis.

Procedure:
Animals are administered the test drug and placed on their backs at regular intervals. The inability of the animal to return to the normal upright posture within a few seconds indicates loss of the righting reflex.

Evaluation:
The **time required for the loss and recovery of the righting reflex** is recorded. A shorter onset and longer duration indicate stronger hypnotic activity.

4. Locomotor Activity Test (Actophotometer)

Principle:
Sedative drugs reduce **spontaneous locomotor activity** by depressing the central nervous system.

Procedure:
Animals are placed in an actophotometer or locomotor activity chamber that records movement through light beams or sensors. After administration of the test compound, the animal's activity is recorded for a specified period.

Evaluation:
A **decrease in locomotor activity counts** compared with the control group indicates sedative or CNS depressant effects.

5. Open Field and Hole Board Tests

Principle:
Sedative drugs decrease **exploratory behavior and spontaneous activity** in animals placed in a novel environment.

Procedure:
Animals are placed in an open field apparatus or a hole board containing several holes on the floor. The number of movements, crossings, and head-dipping activities are recorded after administration of the test compound.

Evaluation:
A **reduction in exploratory movements or head-dipping behavior** compared with control animals indicates sedative activity.

6. Rotarod Performance Test

Principle:
Sedative and hypnotic drugs may impair **motor coordination and balance** due to central nervous system depression.

Procedure:
Animals are trained to remain on a rotating rod (rotarod). After administration of the test drug, the animals are placed on the rotating rod and the time they remain on it is recorded.

Evaluation:
A **decrease in the time the animal remains on the rotating rod** indicates impairment of motor coordination caused by sedative or CNS depressant drugs.

7. Grip Strength and Traction Tests

Principle:
Sedative drugs may produce **muscle relaxation and decreased neuromuscular strength**.

Procedure:
In the grip strength test, animals are allowed to grasp a metal bar connected to a force meter. In the traction test, animals are suspended by their forelimbs on a wire. After administration of the test compound, the ability of the animal to maintain its grip is observed.

Evaluation:
A **reduction in grip strength or inability to hold the wire** indicates muscle relaxation and CNS depressant activity.

8. Exploratory Behavior Tests (Rearing and Head-Dipping)

Principle:
Sedative drugs reduce **spontaneous exploratory behavior** such as rearing and head-dipping in rodents.

Procedure:
Animals are placed in an observation chamber after drug administration. The number of rearing movements or head-dipping responses is recorded over a fixed time period.

Evaluation:
A **decrease in exploratory behaviors** compared with the control group indicates sedative activity.

9. EEG Sleep Analysis

Principle:
Electroencephalography (EEG) records electrical activity in the brain and can identify **different stages of sleep**. Sedative-hypnotic drugs modify EEG patterns associated with sleep.

Procedure:
Electrodes are implanted in the animal's skull to record brain activity. After administration of the test drug, EEG recordings are taken to observe changes in sleep patterns.

Evaluation:
Changes in **sleep onset time, duration of sleep stages, and EEG wave patterns** are analyzed to determine the hypnotic activity of the compound.

ANTIDEPRESSANT MODELS

Depression is a common psychiatric disorder characterized by **persistent low mood, loss of interest or pleasure (anhedonia), reduced motivation, and impaired cognitive function**. The disorder is associated with disturbances in several neurochemical systems of the brain, particularly those involving **monoamine neurotransmitters such as serotonin, norepinephrine, and dopamine**.

Experimental models of depression are important in **preclinical pharmacological research** for the identification and evaluation of new antidepressant drugs. Since the exact pathophysiology of depression in humans is complex and multifactorial, various **behavioral, neurochemical, and stress-based animal models** have been developed to mimic certain features of the disorder.

Many of these models are based on **behavioral despair, chronic stress exposure, or pharmacologically induced monoamine depletion**, which produce depression-like symptoms in animals. These experimental approaches allow researchers to evaluate the **antidepressant activity of compounds by measuring changes in behavior, locomotor activity, stress responses, and neurochemical alterations**.

Classification of Antidepressant Models

Category	Models / Tests
1. Behavioral Despair Models	Forced swim test (Porsolt test), Tail suspension test
2. Learned Helplessness Models	Learned helplessness paradigm in rats or mice
3. Stress-Induced Depression Models	Chronic mild stress model, Chronic unpredictable stress model
4. Neurochemical Models	Reserpine-induced depression model, Tetrabenazine-induced depression
5. Anhedonia Models	Sucrose preference test
6. Locomotor and Behavioral Activity Models	Open field test, Locomotor activity test
7. Neuroendocrine Models	Hypothalamic–pituitary–adrenal (HPA) axis studies
8. In Vitro Neurotransmitter Models	Monoamine uptake inhibition assay, Serotonin and norepinephrine reuptake assays

1. Forced Swim Test (Porsolt Test)

Principle:
The forced swim test is based on the concept of **behavioral despair**. When rodents are placed in an

inescapable container of water, they initially try to escape but eventually adopt an immobile posture. Antidepressant drugs reduce the duration of immobility by increasing active escape behavior.

Procedure:
Mice or rats are placed individually in a cylindrical container filled with water from which they cannot escape. The animals are allowed to swim for a fixed period. Test drugs are administered before the experiment, and the animal's behavior is recorded during the observation period.

Evaluation:
The **duration of immobility** is measured. A **reduction in immobility time** compared with the control group indicates antidepressant activity.

2. Tail Suspension Test

Principle:
This test also measures **behavioral despair**. When mice are suspended by their tails, they initially struggle to escape but eventually remain immobile. Antidepressant drugs decrease the immobility period.

Procedure:
Mice are suspended by the tail using adhesive tape attached to a horizontal bar. The animals are observed for a specific time period after administration of the test compound.

Evaluation:
The **time spent immobile** is recorded. A **significant decrease in immobility duration** indicates potential antidepressant effects.

3. Learned Helplessness Model

Principle:
Repeated exposure to unavoidable stress leads animals to develop **learned helplessness**, a condition resembling depressive behavior. Antidepressant drugs reverse this helplessness.

Procedure:
Animals are exposed to uncontrollable stress such as unavoidable electric shocks. Later they are placed in an apparatus where escape is possible. Test drugs are administered during the experimental period.

Evaluation:
The **number of escape failures** or delayed escape responses are recorded. A **reduction in escape failures** indicates antidepressant activity.

4. Chronic Mild Stress Model

Principle:
Chronic exposure to mild stress produces **depression-like symptoms**, including reduced interest in rewarding stimuli.

Procedure:
Animals are subjected to various mild stressors such as changes in light cycle, food deprivation, or cage tilting over several weeks. Test compounds are administered during or after the stress period.

Evaluation:
Behavioral changes such as **reduced sucrose consumption or decreased activity** are measured. Reversal of these changes indicates antidepressant effects.

5. Reserpine-Induced Depression Model

Principle:
Reserpine depletes **monoamine neurotransmitters (serotonin, norepinephrine, and dopamine)** in the brain, leading to depression-like symptoms.

Procedure:
Animals are administered reserpine to induce behavioral signs such as sedation, hypothermia, and ptosis. Test drugs are then given to evaluate their ability to reverse these symptoms.

Evaluation:
Improvement in **behavioral signs and reversal of reserpine-induced effects** indicates antidepressant activity.

6. Tetrabenazine-Induced Depression Model

Principle:
Tetrabenazine also depletes monoamines in the brain, producing depressive symptoms similar to those seen in reserpine treatment.

Procedure:
Animals receive tetrabenazine to induce depression-like behavior. Test drugs are administered to evaluate their ability to counteract these effects.

Evaluation:
Reduction in **sedation, ptosis, and behavioral depression** compared with control animals indicates antidepressant potential.

7. Sucrose Preference Test (Anhedonia Model)

Principle:
Anhedonia, or loss of pleasure, is a core symptom of depression. Animals normally prefer sweet solutions such as sucrose, but stress-induced depression reduces this preference.

Procedure:
Animals are provided with two bottles containing water and sucrose solution. After exposure to stress or drug treatment, the consumption of each liquid is measured.

Evaluation:
A **decrease in sucrose consumption** indicates anhedonia. Antidepressant drugs restore sucrose preference.

8. Locomotor Activity and Open Field Test

Principle:
Depression and CNS depression may reduce **spontaneous locomotor and exploratory behavior**.

Procedure:
Animals are placed in an open field apparatus or locomotor activity chamber after drug administration. Movements and exploratory behavior are recorded for a fixed period.

Evaluation:
Changes in **locomotor activity and exploratory behavior** are measured. Normalization of activity levels suggests antidepressant effects.

9. Neuroendocrine Models (HPA Axis Studies)

Principle:
Depression is associated with disturbances in the **hypothalamic–pituitary–adrenal (HPA) axis**, which regulates stress hormones such as cortisol.

Procedure:
Animals are exposed to stress or treated with test compounds, and hormone levels such as corticosterone are measured.

Evaluation:
Normalization of **stress hormone levels** indicates potential antidepressant activity.

10. Monoamine Uptake Inhibition Assay

Principle:
Many antidepressant drugs act by **inhibiting the reuptake of monoamine neurotransmitters** such as serotonin and norepinephrine.

Procedure:
Neuronal preparations or synaptosomes are incubated with labeled neurotransmitters and the test compound.

Evaluation:
A **decrease in monoamine uptake** compared with control preparations indicates inhibition of neurotransmitter reuptake and antidepressant activity.

ANTIEPILEPTIC MODELS

Epilepsy is a chronic neurological disorder characterized by **recurrent and unprovoked seizures** resulting from abnormal, excessive, or synchronous neuronal activity in the brain. Seizures may vary in severity and manifestation, ranging from brief lapses in awareness to severe convulsions involving loss of consciousness and motor disturbances.

The pathophysiology of epilepsy involves **imbalance between excitatory and inhibitory neurotransmission** in the central nervous system. Excessive excitatory activity mediated by neurotransmitters such as **glutamate** or reduced inhibitory activity mediated by **gamma-aminobutyric acid (GABA)** can lead to abnormal neuronal firing and seizure generation.

Experimental models of epilepsy are essential in **drug discovery and pharmacological research** for the screening and evaluation of potential antiepileptic agents. These models mimic different types of seizures observed in humans and allow researchers to study the **mechanism of seizure generation and the anticonvulsant activity of drugs**.

Various **electrical, chemical, genetic, and neurophysiological animal models** are used to induce seizures in laboratory animals. These experimental approaches help determine the **efficacy, potency, and mechanism of action** of antiepileptic drugs before they are evaluated in clinical studies

Classification of Antiepileptic Models

Category	Models / Tests
1. Electrical Seizure Models	Maximal electroshock seizure (MES) test
2. Chemical-Induced Seizure Models	Pentylenetetrazol (PTZ) induced seizures, Bicuculline-induced seizures, Strychnine-induced seizures
3. Kindling Models	Amygdala kindling model, Hippocampal kindling model
4. Genetic Epilepsy Models	Genetically epilepsy-prone rats (GEPR), Audiogenic seizure models
5. Status Epilepticus Models	Pilocarpine-induced seizures, Kainic acid-induced seizures
6. Absence Seizure Models	γ-Butyrolactone-induced absence seizures
7. In Vitro Seizure Models	Brain slice electrophysiology models, Hippocampal slice seizure models
8. Neurochemical and Molecular Models	GABA receptor binding assays, Glutamate receptor assays

1. Maximal Electroshock Seizure (MES) Test

Principle

The maximal electroshock seizure model is based on the induction of **generalized tonic–clonic seizures** by an electrical stimulus applied to the brain. Antiepileptic drugs that inhibit the **spread of seizure activity** can prevent the characteristic **tonic hind limb extension** phase produced by electroshock.

Procedure

Healthy adult mice or rats are selected and divided into control, standard, and test groups. The animals are maintained under standard laboratory conditions with free access to food and water. The test compound is administered orally or intraperitoneally. A standard anticonvulsant drug such as phenytoin is given to the standard group.

After a pretreatment period of about 30 minutes, seizures are induced using an electroconvulsiometer. Electrodes are placed on the **cornea or ears** of the animal after applying saline to improve electrical conduction. A current of about **50–60 Hz for 0.2–0.3 seconds** is delivered.

This electrical stimulation produces a sequence of convulsive phases including **tonic flexion, tonic hind limb extension, clonic convulsions, stupor, and recovery**. Each animal is observed carefully after the stimulus.

Evaluation

The main parameter observed is the **presence or absence of tonic hind limb extension**. Animals that do not show tonic extension are considered protected. The **percentage protection** is calculated to determine anticonvulsant activity.

2. Pentylenetetrazol (PTZ)-Induced Seizure Model
Principle

Pentylenetetrazol produces seizures by **blocking GABA-mediated inhibitory neurotransmission**, leading to neuronal hyperexcitability. Drugs that enhance inhibitory transmission or reduce excitatory activity can prevent PTZ-induced seizures.

Procedure

Mice or rats are divided into control, standard, and test groups. The test compound is administered through oral or intraperitoneal routes. A standard drug such as diazepam or sodium valproate is used.

After about **30 minutes of pretreatment**, PTZ is injected intraperitoneally at a convulsive dose (approximately **60–80 mg/kg in mice**). Immediately after injection, animals are placed in individual observation cages.

Animals are monitored continuously for signs of **tremors, jerking movements, clonic convulsions, tonic seizures, and loss of posture**.

Evaluation The latency to onset of seizures**,** duration of seizures**, and** mortality rate **are recorded. Drugs that** delay seizure onset or prevent seizures **show anticonvulsant activity.**

3. Bicuculline-Induced Seizure Model
Principle

Bicuculline induces seizures by **blocking GABA receptors**, which reduces inhibitory neurotransmission and causes neuronal hyperexcitability.

Procedure

Animals are divided into control and treated groups. The test compound is administered before seizure induction. After the pretreatment period, **bicuculline** is injected intraperitoneally at a convulsive dose.

Animals are placed in observation cages and monitored for convulsive signs such as **tremors, clonic seizures, and tonic convulsions**.

Evaluation

The **time to seizure onset and severity of convulsions** are recorded. Drugs that delay the onset or reduce the severity of seizures demonstrate anticonvulsant activity.

4. Strychnine-Induced Seizure Model
Principle

Strychnine causes seizures by **blocking glycine receptors in the spinal cord**, leading to increased neuronal excitation.

Procedure

Animals are divided into groups and treated with the test compound before seizure induction. After the pretreatment period, **strychnine** is administered subcutaneously or intraperitoneally at a convulsive dose.

Animals are placed in transparent cages and observed for signs of convulsions such as **muscle rigidity, tonic spasms, and respiratory distress**.

Evaluation

The **latency to convulsions and survival time** are recorded. Compounds that delay seizure onset or prevent death show anticonvulsant activity.

5. Kindling Model (Amygdala or Hippocampal Kindling)
Principle

Repeated electrical stimulation of brain regions such as the **amygdala or hippocampus** gradually produces a permanent epileptic condition called **kindling**.

Detailed Procedure

Rats are anesthetized and electrodes are surgically implanted in the amygdala or hippocampus. After recovery, animals receive repeated electrical stimulations using a stimulator.

The stimulation is applied once daily over several days or weeks. Initially mild responses occur, but repeated stimulation leads to **generalized seizures**.

Test compounds are administered during the kindling process.

Evaluation

The **severity, frequency, and progression of seizures** are recorded. Drugs that reduce seizure severity or delay kindling development are considered effective.

6. Genetic Epilepsy Model (Audiogenic Seizures)
Principle

Some rodents have a **genetic predisposition to seizures triggered by loud sound**.

Procedure

Animals are placed in a sound-proof chamber equipped with a loudspeaker. After administration of the test compound, animals are exposed to a **high-intensity sound stimulus**.

The sound stimulus triggers seizure activity such as **wild running, clonic convulsions, and tonic seizures**.

Evaluation

The **incidence and severity of seizures** are recorded. Reduction in seizure frequency indicates anticonvulsant activity.

7. Status Epilepticus Model (Pilocarpine or Kainic Acid)
Principle

Pilocarpine and kainic acid induce **prolonged seizures (status epilepticus)** by activating excitatory neurotransmitter pathways in the brain.

Procedure

Animals are divided into control and treatment groups. The test compound is administered before seizure induction. **Pilocarpine or kainic acid** is injected intraperitoneally.

Animals are observed continuously for behavioral signs such as **tremors, salivation, clonic convulsions, and tonic seizures**.

Evaluation

The **latency, duration, and severity of seizures** are recorded. Drugs that reduce seizure severity or prevent prolonged seizures show anticonvulsant activity.

8. Absence Seizure Model (γ-Butyrolactone)
Principle

γ-Butyrolactone induces **absence seizures** characterized by brief behavioral arrest and specific EEG patterns.

Procedure

Animals are divided into control and treated groups. The test compound is administered prior to seizure induction. **γ-Butyrolactone** is injected intraperitoneally to produce absence seizures.

Animals are observed for episodes of **behavioral arrest, twitching, or staring behavior**.

Evaluation

The **frequency and duration of seizure episodes** are measured. Drugs that reduce these episodes show effectiveness against absence seizures.

9. In Vitro Seizure Model (Brain Slice or Hippocampal Slice)
Principle

Isolated brain tissue can produce **epileptiform electrical activity**, which allows evaluation of anticonvulsant drugs in controlled conditions.

Procedure

Animals are anesthetized and the brain is removed quickly. Thin slices of brain tissue, particularly from the **hippocampus**, are prepared and placed in oxygenated physiological solution.

Electrical or chemical stimulation is applied to induce epileptiform activity. Test compounds are added to the solution.

Evaluation

Changes in **neuronal electrical activity and epileptiform discharges** are recorded. Suppression of abnormal activity indicates anticonvulsant potential.

10. Neurochemical and Molecular Models (GABA and Glutamate Receptor Assays)
Principle

Many antiepileptic drugs act by **enhancing GABAergic inhibition or reducing glutamatergic excitation**.

Procedure

Brain tissue or receptor preparations are incubated with radiolabeled ligands that bind to **GABA or glutamate receptors**. Test compounds are added to evaluate their interaction with these receptors.

Biochemical assays are used to measure receptor binding or neurotransmitter levels.

Evaluation

Changes in **receptor binding or neurotransmitter activity** indicate the mechanism of anticonvulsant action.s

ANTIPSYCHOTIC MODELS

Antipsychotic drugs are used in the treatment of **psychotic disorders**, particularly **Schizophrenia**, which is characterized by disturbances in thought, perception, emotion, and behavior. Symptoms of schizophrenia are generally classified into **positive symptoms** such as hallucinations, delusions, and agitation, and **negative symptoms** such as social withdrawal, reduced motivation, and emotional blunting.

The development of antipsychotic drugs is based mainly on the **dopamine hypothesis of schizophrenia**, which suggests that excessive activity of **dopaminergic neurotransmission**, particularly through **dopamine D_2 receptors**, plays an important role in the development of psychotic symptoms. Many conventional antipsychotic drugs exert their therapeutic effect by **blocking dopamine D_2 receptors in the brain**.

Experimental animal models are widely used in drug discovery to evaluate the **antipsychotic potential of new compounds**. These models reproduce certain behavioral and neurochemical alterations associated with psychosis. Pharmacological agents such as dopamine agonists or NMDA receptor antagonists are used to induce behavioral abnormalities in animals that resemble symptoms of psychosis.

Therefore, antipsychotic screening models nclude **dopamine agonist–induced behavioral tests, conditioned avoidance response models, catalepsy tests, locomotor activity models, and neurochemical receptor assays**. These experimental models are important for assessing the **efficacy, mechanism of action, and safety of potential antipsychotic drugs** before they proceed to clinical evaluation.

Classification of Antipsychotic Models

Category	Animal Models / Tests
1. Dopamine Agonist-Induced Models	Apomorphine-induced stereotypy, Amphetamine-induced hyperactivity, Apomorphine-induced climbing behavior in mice
2. Conditioned Avoidance Response (CAR) Models	Conditioned avoidance response test in rats
3. Catalepsy Models	Haloperidol-induced catalepsy, Bar test for catalepsy
4. Prepulse Inhibition (PPI) Models	Disruption of prepulse inhibition of acoustic startle response
5. Locomotor Activity Models	Amphetamine-induced locomotor activity test, Open-field locomotor activity test
6. Social Interaction Models	Social interaction test in rodents
7. NMDA Antagonist-Induced Psychosis Models	Phencyclidine (PCP)-induced hyperactivity, Ketamine-induced behavioral changes
8. Neurochemical and Receptor Binding Models	Dopamine D2 receptor binding assays, Serotonin (5-HT2A) receptor assays
9. In Vitro Models	Dopamine uptake inhibition assay, Receptor binding assays

1. Apomorphine-Induced Stereotypy

Principle

Apomorphine is a **dopamine receptor agonist** that stimulates dopaminergic pathways in the brain and produces stereotyped behaviors such as sniffing, licking, and gnawing in rodents. Antipsychotic drugs that **block dopamine D_2 receptors** inhibit these stereotyped behaviors.

Procedure

Mice or rats are divided into control, standard, and test groups. The animals are maintained under standard laboratory conditions. The test compound is administered orally or intraperitoneally. A known antipsychotic drug such as haloperidol is used as the standard drug.

After a pretreatment period of about **30–60 minutes**, apomorphine is injected subcutaneously at a dose that produces stereotyped behavior. Animals are placed individually in observation cages.

Behavioral changes such as **continuous sniffing, licking, biting, and gnawing movements** are carefully observed for a specific time period (usually about 30–60 minutes).

Evaluation

The **intensity and frequency of stereotyped behavior** are recorded using a behavioral scoring system. Drugs that significantly **reduce stereotypy scores** are considered to possess antipsychotic activity.

2. Amphetamine-Induced Hyperactivity

Principle

Amphetamine increases **dopamine release in the brain**, resulting in increased locomotor activity and behavioral excitation. Antipsychotic drugs reduce this hyperactivity by **blocking dopaminergic transmission**.

Detailed Procedure

Animals are divided into control, standard, and test groups. The test compound is administered before amphetamine administration. After the pretreatment period, **amphetamine** is injected intraperitoneally at a dose that produces hyperactivity.

Animals are placed in activity cages or actophotometers to measure locomotor movements.

Evaluation

The **number of movements or activity counts** is recorded for a specific time period. A significant **reduction in amphetamine-induced hyperactivity** indicates antipsychotic activity.

3. Apomorphine-Induced Climbing Behavior in Mice

Principle

Apomorphine stimulates dopamine receptors and produces **climbing behavior in mice placed in wire mesh cages**. Antipsychotic drugs block dopamine receptors and reduce this climbing response.

Detailed Procedure

Mice are placed in cylindrical cages with wire mesh walls. The test compound is administered prior to apomorphine injection.

After the pretreatment interval, apomorphine is injected subcutaneously. Animals are observed for climbing activity such as **clinging to the cage walls**.

Evaluation

The **time spent climbing and number of climbing episodes** are recorded. Drugs that reduce climbing behavior are considered effective antipsychotic agents.

4. Conditioned Avoidance Response (CAR) Test

Principle

In this model, animals learn to avoid a mild electric shock when they receive a warning signal such as light or sound. Antipsychotic drugs **selectively suppress avoidance behavior without affecting escape behavior**.

Detailed Procedure

Rats are trained in a shuttle box with two compartments separated by a barrier. A **conditioned stimulus** such as light or sound is given before a mild electric shock is applied to the floor.

During training, the rat learns to move to the other compartment to avoid the shock. After successful training, the test compound is administered.

The effect of the drug on avoidance responses is observed.

Evaluation

The **number of avoidance responses and escape responses** are recorded. Antipsychotic drugs typically **reduce avoidance responses while preserving escape responses**.

5. Catalepsy Test (Haloperidol-Induced Catalepsy / Bar Test)

Principle

Catalepsy is a condition of **muscular rigidity and immobility** produced by dopamine receptor blockade in the brain. Many typical antipsychotic drugs produce catalepsy in animals.

Detailed Procedure

Animals receive the test compound or standard drug. In the **bar test**, the forepaws of the animal are placed on a horizontal bar elevated above the floor.

The time during which the animal maintains this abnormal posture without correcting it is measured.

Evaluation

The **duration of maintained posture** is recorded. A longer duration indicates stronger dopamine receptor blockade. This test helps evaluate **extrapyramidal side effects of antipsychotic drugs**.

6. Prepulse Inhibition (PPI) Model

Principle

Prepulse inhibition refers to the **reduction in startle response when a weak stimulus precedes a strong stimulus**. In psychotic disorders this mechanism is impaired. Antipsychotic drugs restore normal PPI.

Detailed Procedure

Animals are placed in startle chambers equipped with sensors. A **weak prepulse stimulus** is given shortly before a loud acoustic stimulus.

The startle response is measured using recording devices.

Evaluation

The **magnitude of the startle response** is recorded. Drugs that restore **normal prepulse inhibition** are considered to have antipsychotic potential.

7. Open-Field Locomotor Activity Test

Principle

Psychostimulant drugs increase locomotor activity through dopaminergic stimulation. Antipsychotic drugs reduce excessive locomotion.

Detailed Procedure

Animals are placed in an **open-field apparatus** consisting of a square arena marked with grids. The test compound is administered prior to observation.

Animals are allowed to explore the arena for a fixed time period.

Evaluation

Parameters such as **number of squares crossed, rearing behavior, and exploratory activity** are recorded. A decrease in hyperactivity indicates antipsychotic action.

8. NMDA Antagonist-Induced Psychosis Model (PCP or Ketamine)

Principle

NMDA receptor antagonists such as **phencyclidine (PCP)** or **ketamine** produce behavioral changes similar to symptoms of schizophrenia. Antipsychotic drugs can reduce these abnormalities.

Detailed Procedure

Animals receive the test compound before administration of PCP or ketamine. These agents are injected intraperitoneally to induce behavioral disturbances such as **hyperlocomotion and stereotypy**.

Animals are then observed in activity cages.

Evaluation

The **degree of hyperactivity, stereotypy, and behavioral disturbances** is recorded. Drugs that reduce these symptoms demonstrate antipsychotic activity.

9. Dopamine D_2 and Serotonin (5-HT_2A) Receptor Binding Assays

Principle

Many antipsychotic drugs act by **blocking dopamine D_2 receptors and serotonin 5-HT_2A receptors**.

Detailed Procedure

Brain tissue preparations containing these receptors are incubated with **radiolabeled ligands**. The test compound is added to the incubation mixture to evaluate its ability to compete with the ligand for receptor binding.

Evaluation

The **degree of inhibition of ligand binding** is measured. High receptor affinity suggests potential antipsychotic activity.

10. Dopamine Uptake Inhibition Assay (In Vitro)

Principle

This model evaluates the ability of compounds to influence **dopamine transport mechanisms** in neuronal tissue.

Detailed Procedure

Synaptosomal preparations from brain tissue are incubated with **radiolabeled dopamine**. Test compounds are added to observe their effect on dopamine uptake into nerve terminals.

Evaluation

The **amount of dopamine uptake inhibition** is measured. Compounds affecting dopamine transport may possess antipsychotic activity.

IMPORTANT QUESTIONS

Very Short Questions (2 marks each)

1. What is the importance of dose selection in preclinical studies?
2. Define sham control in animal studies.
3. What is a positive control group?
4. Mention one reason for selecting a specific animal species for a study.
5. Name one screening model for diuretics.
6. What is the role of nootropic drugs?
7. Define the term "analgesic."
8. What is the purpose of an anti-inflammatory drug?
9. Name one animal model used for testing antipsychotic drugs.
10. What is an anti-Parkinson's drug?

Short Questions (5 marks each)

1. Explain the process of dose calculation and conversions in preclinical studies.
2. Describe the preparation of drug solutions/suspensions for animal studies.
3. Discuss the importance of using negative and positive control groups in preclinical screening.
4. Illustrate the rationale for selecting the sex of animals in a study.
5. Describe an animal model used for screening anti-asthmatic drugs.
6. Explain the screening model for testing the analgesic activity of a drug.
7. Summarize the method of evaluating sedative and hypnotic drugs in preclinical models.
8. Discuss the significance of antipyretic screening models.
9. Describe an animal model used for screening antidepressant drugs.
10. Explain the screening process for anti-epileptic drugs.

Long Questions (10 marks each)

1. Analyze the importance of proper dose selection, calculation, and conversion in preclinical studies.
2. Discuss the preparation of drug solutions/suspensions and its significance in preclinical animal studies.
3. Evaluate the role of sham, negative, and positive control groups in ensuring the reliability of preclinical studies.
4. Critically assess the rationale for the selection of animal species and sex in preclinical research.
5. Describe the comprehensive screening model for diuretics, including methodology and expected outcomes.
6. Discuss the preclinical screening models for nootropics, highlighting the procedures and significance.
7. Analyze the screening models for anti-Parkinson's drugs, focusing on the mechanisms and evaluation criteria.
8. Compare and contrast different animal models used for screening anti-asthmatic drugs.
9. Evaluate the various preclinical screening models for CNS activity, including analgesic, antipyretic, and anti-inflammatory drugs.
10. Discuss the ethical and practical challenges in the preclinical screening of general anesthetics, sedatives, hypnotics, antipsychotics, and antidepressants.