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🧪 Preclinical Screening of New Substance for Pharmacological Activity

🔷 What is Preclinical Screening?

Preclinical screening is the **testing of a new drug or substance** (before giving it to humans) to check:

- Is it **safe**?
- Does it **work**?
- What are its **side effects**?

This is done using **laboratory animals**, **cells**, or **non-animal methods**.

⚙️ General Principles of Preclinical Screening

1. ✅ **Safety First**: The substance must not be harmful to cells, organs, or animals.
2. ✅ **Effectiveness**: The drug should show the desired pharmacological action (e.g., reduce pain, lower blood pressure).
3. ✅ **Right Dose**: Identify the correct and safe dose range.
4. ✅ **Route of Administration**: Test how the drug behaves when given orally, by injection, etc.
5. ✅ **Reproducibility**: Results should be consistent and repeatable.
6. ✅ **Ethical Use of Animals**: Follow animal welfare rules (3Rs – Replace, Reduce, Refine).

🐁 1. In Vivo Models (Animal Testing)

"In vivo" means testing inside a living animal.

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🔍 **Purpose:**

- To study the **effect of the substance on the whole body** (organs, behavior, blood, etc.).

🧪 Common In Vivo Tests:

Test Type	Purpose	Common Animals Used
Acute toxicity test	Check safety after one large dose	Mice, rats
Anti-inflammatory test	Check swelling reduction	Rats (carrageenan test)
Analgesic (pain relief)	Pain reduction (hot plate test)	Mice, rats
Antidepressant screening	Behavior tests (forced swim test)	Mice, rats
Antidiabetic screening	Blood sugar reduction	Diabetic rats, mice
Cardiovascular tests	Heart and BP studies	Dogs, rabbits, pigs

🧫 2. In Vitro Models (Outside the Body – Test Tube or Cell Models)

"In vitro" means testing outside the living body, usually in test tubes, petri dishes, or well plates.

🔍 **Purpose:**

- To test how a drug affects **cells, tissues, or isolated organs**.

□ Common In Vitro Methods:

Method	Use Example
Cell culture models	Cancer drug testing on tumor cells
Isolated tissue studies	Muscle/nerve contraction testing
Enzyme inhibition tests	Study effect on enzymes like ACE
Receptor binding assays	Study how drug binds to receptors

✅ Advantages:

- No animals used.
- Cheaper and faster.
- Can test many drugs at once.

🧬 3. Alternative Models to Animals

In preclinical research, animals are often used to test new drugs. But to reduce animal use, scientists have developed alternative models. These models help in Replacing, Reducing, or Refining animal use (known as the 3Rs principle).

📌 1. In Vitro Models (Test in the lab using cells)

These are tests done outside the body, using cells or tissues grown in the lab.

🔹 Cell Culture:

- **Uses human or animal cells in a dish.**
- **Example: Liver cells (HepG2) to test liver toxicity.**

🔹 3D Cell Culture:

- More realistic than simple cell layers.
- Mimics actual tissues.

📌 2. In Silico Models (Computer-based testing)

These use computers to predict how a drug will behave in the body.

🔹 QSAR (Quantitative Structure-Activity Relationship):

- Predicts a chemical's activity based on its structure.

🔹 Molecular Docking:

- Checks how a drug binds to a target protein.

📌 3. Organ-on-a-Chip

These are small devices with human cells that act like real organs.

- Example: Lung-on-a-chip to test asthma drugs.
- Helps study real organ functions without animals.

📌 4. Stem Cell-Based Models

Human stem cells are grown into tissues (like heart or brain cells) to study drug effects.

- Example: Heart cells to test for heart toxicity.

📌 5. Zebrafish Embryos

Zebrafish embryos are transparent and grow quickly.

- Used to test effects on development and organ damage.

- **Considered an alternative because they are not fully developed animals in early stages.**

📌 6. High-Throughput Screening (HTS)

Uses robots and machines to test many drugs quickly using cell-based tests.

- **Fast and does not use animals.**

✅ Benefits of Alternative Models

- **Less use of animals**
- **Lower cost**
- **Faster results**
- **Better for human-specific reactions**

🔄 Flow of Preclinical Screening (General Steps)

1. **In vitro tests** → check effect on cells/tissues
2. **In vivo tests** → confirm effect on whole animal
3. **Toxicity studies** → find safe dose, side effects
4. **Alternative models** → used wherever possible
5. **Approval for clinical trial** → if safe, tested in humans

⚖️ Ethical Considerations: 3Rs Principle

- **Replace**: Use non-animal methods when possible.
- **Reduce**: Use minimum number of animals.

- **Refine**: Minimize pain or distress in animals.

□ CNS Pharmacology: Behavioral and Muscle Coordination

◈ What is CNS Pharmacology?

CNS pharmacology is the study of how drugs affect the **central nervous system** (brain and spinal cord). It includes:

- Changes in **behavior**
- Changes in **movement**
- **Mood**, **sleep**, **alertness**, and **muscle control**

🧍‍♂️ 1. Behavioral Studies

These tests observe **how a drug changes the animal's behavior**, including activity, anxiety, sleep, and alertness.

✅ Common Behavioral Tests:

Test Name	Purpose	Animal Used	Example Outcome
Open Field Test	Measures activity & anxiety	Mice, rats	More movement = hyperactivity
Elevated Plus Maze	Measures anxiety (open vs closed arms)	Mice, rats	More time in open = less anxiety
Forced Swim Test	Measures depression-like behavior	Mice, rats	Less struggle = depression-like

Test Name	Purpose	Animal Used	Example Outcome
Hole Board Test	Checks curiosity and exploration	Mice, rats	Head dips = exploratory behavior
Rotarod Test	Measures coordination & balance	Mice, rats	More time on rod = better balance

💪 2. Muscle Coordination Studies

These tests check **how drugs affect muscle strength, balance, and coordination**. They are important in **neurological drug testing** (e.g., for Parkinson's, sedatives, muscle relaxants).

✅ Common Muscle Coordination Tests:

Test Name	Purpose	Animal Used	Observations
Rotarod Test	Balance and motor coordination	Mice, rats	Time animal stays on rotating rod
Grip Strength Test	Measures muscle strength	Mice, rats	How hard the animal pulls a bar
Actophotometer Test	Measures general locomotor activity	Mice, rats	Light beams broken = movement
Inclined Plane Test	Tests ability to hold position	Rats	Angle of slip = muscle tone
Bar Test	Tests muscle rigidity (catalepsy)	Rats, mice	Measures sedative or antipsychotic effects

💊 **Why These Tests Are Used:**

- To study the **effect of CNS drugs** like:
  - **Stimulants** (increase activity)
  - **Sedatives** (calm or reduce activity)
  - **Anxiolytics** (reduce anxiety)
  - **Antidepressants**
  - **Antipsychotics**
  - **Muscle relaxants**
- To evaluate **side effects** like drowsiness, poor coordination, or muscle weakness.

⚖️ **Ethical Note:**

- Always follow **ethical guidelines** (like IAEC/CPCSEA).
- Use **minimum number of animals**.
- Ensure **animal comfort** and minimize suffering.

CNS Stimulants & Depressant

CNS Stimulants are substances that increase brain activity. They enhance alertness, attention, energy, and physical activity by increasing the levels of certain neurotransmitters such as dopamine and norepinephrine. Common examples include caffeine, amphetamines, and cocaine. These drugs are often used to treat

conditions like attention-deficit hyperactivity disorder (ADHD) and narcolepsy.

- Preclinical Screening for Central Nervous System Stimulants

- Some screening methods for CNS stimulant drugs are described as follows:

In vivo methods

(i) Sandauswurf" (displacement of sand) method
(ii) Runway test
(iii) Ptosis test
(iv) Registration of motor activity
(v) Open field test
(vi) Hole-board test
(vii) Combined open field test

□ Runway Test

🎯 AIM:

To evaluate the effect of **CNS active drugs** (mainly **CNS stimulants and depressants**) on **locomotor activity**, **learning**, and **motivation** in experimental animals (usually rats or mice) using a **runway apparatus**.

□ REQUIREMENTS:

- Experimental animal (rat or mouse)

- Runway apparatus (long tunnel with start and goal box)
- Reward (food or water) or avoidance stimulus (like mild shock)
- Stopwatch or timer
- Test drug (e.g., stimulant like amphetamine or depressant like diazepam)

PROCEDURE:

1. **Acclimatization:**
   - Allow the animal to get used to the runway box for 1–2 days without any drug.
   - No shocks or rewards during this time.
2. **Training Phase:**
   - Place the animal in the **start box**.
   - The **goal box** contains a reward (e.g., food or water) or helps the animal avoid an unpleasant stimulus.
   - Let the animal **run** from start to goal.
   - Record the **time taken** to reach the goal (called **latency**).
3. **Test Phase (After Drug Administration):**
   - Give the animal the **test drug** (stimulant or depressant).
   - After the drug takes effect (usually 30–60 minutes), place the animal again in the start box.
   - Record **time taken** to reach the goal box after drug treatment.
4. **Repeat the test** for a few days to check for **changes in speed, motivation, or memory**.

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📋 OBSERVATIONS:

- **CNS Stimulants** (e.g., amphetamine): Reduce the time to reach the goal (increased activity).
- **CNS Depressants** (e.g., diazepam): Increase the time or cause the animal to stop (reduced activity or motivation).
- **Learning drugs**: Improve the animal's ability to reach the goal faster over time.

✅ CONCLUSION:

The runway test helps determine how a drug **affects CNS activity**, **motor coordination**, **motivation**, and sometimes **learning and memory**:

- Faster performance = **CNS stimulation or improved learning**
- Slower performance = **CNS depression or sedation**

□ Open Field Test

🎯 AIM:

To assess the **general activity level**, **exploratory behavior**, and **anxiety levels** of experimental animals (usually rodents like rats or mice) in response to CNS-active drugs.

This test helps in evaluating the **psychotropic effects** of **CNS stimulants** (increased activity) and **CNS depressants** (reduced activity).

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▯ REQUIREMENTS:

- **Experimental animals** (mice or rats)
- **Open field apparatus**: A large, enclosed box (usually 50 cm x 50 cm or larger), with a flat floor and high walls, often with grids on the floor for tracking movement.
- **Recording system** (optional): Camera or activity meter to record movement.
- **Test drug** (CNS stimulant or depressant)

▯ PROCEDURE:

1. **Acclimatization:**
    - Place the animal in the open field for **5-10 minutes** without any drug, to allow it to adjust to the environment.
    - This step helps the animal become familiar with the surroundings and reduces initial anxiety.
2. **Drug Administration:**
    - Administer the **test drug** (CNS stimulant like amphetamine or depressant like diazepam) to the animal.
    - Allow the animal to **rest for 30–60 minutes** after administration so the drug can take effect.
3. **Testing:**
    - Place the animal back in the open field and allow it to **explore** freely for about **10-15 minutes**.
    - Record the animal's **movement**, focusing on:
        - **Total distance traveled** (locomotor activity)

- **Time spent in the center** (exploratory behavior, anxiety)
- **Time spent along the walls** (thigmotaxis, anxiety behavior)
- **Rearing** (standing on hind legs, showing exploration)
- **Frozen behavior** (indicating fear or anxiety)

4. **Repeat** the test for multiple animals, comparing data from animals treated with the drug and those treated with a **placebo** or **control substance**.

📋 **OBSERVATIONS:**

- **CNS Stimulants** (e.g., amphetamines or caffeine):
  - Increase **locomotor activity**, with the animal covering more distance and spending more time exploring the center.
  - **Less thigmotaxis** (less wall-hugging behavior).
- **CNS Depressants** (e.g., diazepam or barbiturates):
  - Reduce **activity levels** (shorter distance traveled).
  - **More time spent near the walls** or **frozen behavior** (anxiety, sedation).
- **Anxiolytics** (e.g., benzodiazepines) may show **increased center exploration** and **decreased thigmotaxis**.

📝 **Summary Table:**

Drug Type	Expected Effect	Observations
CNS Stimulants	Increased activity	More distance covered, less time near walls
CNS Depressants	Decreased activity	Less movement, more wall-hugging, freezing
Anxiolytics	Reduced anxiety, increased exploration	More time in the center, less wall-hugging

In Vitro Tests for CNS Stimulants

🎯 **AIM:**

To assess the **pharmacological activity** of **CNS stimulants** (e.g., amphetamines, caffeine) using **in vitro models**. These tests help to understand how stimulants affect brain cells, neurotransmitter release, and receptor activity without using live animals.

REQUIREMENTS:

- **Cell cultures** (neurons, glial cells, or brain slices)
- **Neurotransmitter assays** (to measure chemicals like dopamine, serotonin, etc.)
- **Receptor binding assays** (to check stimulant interaction with brain receptors)
- **Electrophysiological equipment** (for measuring electrical activity in neurons)
- **Drugs** (CNS stimulants like amphetamines, caffeine, nicotine)

- **Buffers and reagents** for cell culture and testing

□ COMMON IN VITRO TESTS FOR CNS STIMULANTS

1. Neurotransmitter Release Assay

Purpose:

To test how a CNS stimulant affects the **release of neurotransmitters** (like dopamine, norepinephrine, serotonin) from nerve cells.

Procedure:

1. **Culture neurons** in a petri dish.
2. Treat the cells with the **CNS stimulant**.
3. Measure the **increase in neurotransmitter levels** in the media (e.g., dopamine or serotonin) using specific assays (e.g., ELISA or HPLC).
4. Compare the data with **control samples** (no drug).

Expected Results:

- CNS stimulants like **amphetamine** can **increase dopamine release**.
- The more the stimulant increases neurotransmitter release, the more **potent** the drug.

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2. Receptor Binding Assay

Purpose:

To determine if a CNS stimulant **binds to specific receptors** (e.g., dopamine, adrenergic, serotonin receptors) that are involved in CNS activation.

Procedure:

1. Prepare a **membrane preparation** or use **cell lines** that express specific CNS receptors.
2. **Label the drug** (e.g., radiolabel it) and add it to the receptor preparation.
3. Allow the **drug** to bind to the receptors.
4. Measure how much of the drug **binds** to the receptors (using radioactivity or fluorescence).
5. Compare with a **known antagonist** or **competitive inhibitor** to see if the stimulant has an effect.

Expected Results:

- If the CNS stimulant **binds to dopamine receptors**, this suggests that the stimulant works through dopamine pathways to increase alertness or activity.

📌 **Conclusion:**

- In vitro tests for CNS stimulants provide valuable information on how a drug affects neurotransmitter release, receptor binding, and neuronal activity.
- These tests help **reduce animal usage** and understand the **mechanisms of CNS stimulants** at the cellular and molecular level.

Screening Methods for Anxiolytic Activity-

- ✓ Anxiety is a subjective human phenomenon and except for some of the associated somatic and autonomic changes, it has no obvious part in experimental animals.
- ✓ In biological terms anxiety may be regarded as a particular farm of behavioural inhibition that occurs in response to environmental events that are novel, non-rewarding or punishing.
- ✓ In animals this behavioural inhibition may take pressing to obtain food. To develop new and more effective to have animal tests that give a good prediction at activity in man and considerable effort has one into developing and validating such tests.

✅ 1. In Vivo Models for Anxiolytics

(*Tested on live animals like rats or mice*)

🐁 A. Elevated Plus Maze (EPM)

🔹 Aim:

To test if a drug reduces anxiety by checking the animal's willingness to explore open arms of a maze.

🔹 Procedure:

- Use a plus-shaped maze elevated from the ground with 2 open arms and 2 closed arms.
- Place the mouse in the center.
- Let it explore for 5 minutes.
- Record:
  - Time spent in **open arms**

- Number of **entries into open arms**

◈ **Conclusion:**
If the animal spends **more time in open arms**, the drug likely has **anxiolytic (anti-anxiety)** effects.

🐁 B. Light-Dark Box Test

◈ **Aim:**
To check if a drug reduces anxiety by encouraging the animal to enter and stay in a bright area.

◈ **Procedure:**

- Use a box divided into a **dark chamber** and a **light chamber**.
- Place the animal in the **dark side**.
- Let it move freely for 5–10 minutes.
- Record:
  - Time spent in **light side**
  - Number of **crossovers** between light and dark

◈ **Conclusion:**
An anxiolytic drug will cause the animal to **spend more time in the light area** (less fearful, less anxious).

🧫 2. In Vitro Models for Anxiolytics

(*Done in lab dishes using cells or tissues*)

🧫 A. GABA Receptor Binding Assay

◈ **Aim:**
To find out if the drug binds to **GABA-A receptors**, which are involved in calming the brain.

◈ **Procedure:**

- Use brain tissue or cell membranes that contain **GABA-A receptors**.
- Add a **radioactive or fluorescent marker** that binds to the receptor.
- Add the test drug and measure **how much it displaces the marker**.
- The more it binds, the more it interacts with the receptor.

◈ **Conclusion:**
If the drug binds strongly to GABA-A receptors, it may act as an **anxiolytic** by enhancing calming signals in the brain.

B. Neurotransmitter Release from Cultured Neurons

◈ **Aim:**
To study if the drug changes levels of brain chemicals like **GABA** or **serotonin**, which affect anxiety.

◈ **Procedure:**

- Grow **neurons in a petri dish** (from brain tissue).
- Add the drug to the culture.
- Measure the levels of **GABA, serotonin**, or **glutamate** released using chemical tests (e.g., ELISA, HPLC)
-

◈ **Conclusion:**
If the drug **increases GABA** or **reduces excitatory signals**, it shows potential **anti-anxiety** effects.

Antipsychotics

Antipsychotics are **medications** used to **treat mental health conditions** such as **schizophrenia**, **bipolar disorder**, and **severe depression**. They help to **manage symptoms** like **hallucinations**, **delusions**, and **confused thinking** by affecting chemicals in the brain, particularly **dopamine**.

✅ In Vivo Models (Animal-Based Tests)

🐁 1. Apomorphine-Induced Climbing Behavior (Mouse Test)

◈ **Aim:**
To assess **dopamine blocking** (antipsychotic-like) activity of a drug.

◈ **Procedure:**

- Mice are injected with **apomorphine** (a dopamine agonist), which causes **climbing behavior**.
- Then, the test drug (possible antipsychotic) is given.
- Observe and score how much the mouse climbs.

◈ **Conclusion:**
If the drug **reduces climbing**, it suggests **dopamine receptor blocking**, meaning it may have **antipsychotic properties**.

🧠 2. Amphetamine-Induced Stereotypy Test

🔹 **Aim:**
To evaluate drugs that **reduce hyperactivity and repetitive movements** caused by excess dopamine.

🔹 **Procedure:**

- Rats are given **amphetamine**, which causes **stereotyped behaviors** (like sniffing, licking, head bobbing).
- The test drug is given before or after amphetamine.
- Observe the intensity and duration of stereotyped behavior.

🔹 **Conclusion:**
If the drug **reduces stereotypy**, it may be effective as an **antipsychotic**, especially for **positive symptoms** of schizophrenia.

🧪 In Vitro Models (Lab/Cell-Based Tests)

🧫 1. D2 Receptor Binding Assay

🔹 **Aim:**
To test if a drug **binds to dopamine D2 receptors**, which are key targets for antipsychotics.

🔹 **Procedure:**

- Prepare brain tissue or cell membranes containing **D2 receptors**.
- Add a **radio-labeled dopamine-like substance**.
- Add the test drug to see if it **competes for binding**.
- Measure how much binding is reduced.

◈ **Conclusion:**
If the drug **binds well to D2 receptors**, it could block dopamine — indicating **antipsychotic potential**.

□ 2. Calcium Imaging or Signal Transduction Assay

◈ **Aim:**
To check how the drug **affects dopamine signaling pathways** in brain cells.

◈ **Procedure:**

- Use cultured neurons or receptor-expressing cells.
- Add dopamine to activate signaling (e.g., calcium release, cAMP production).
- Then add the test drug to see if it **blocks or alters the response**.
- Use fluorescent dyes or biosensors to measure the change.

◈ **Conclusion:**
If the drug **reduces dopamine signaling**, it may work as an **antipsychotic** by calming overactive brain pathways.

Screening Methods for Anti Epileptic Activity-

Epilepsy is common disorder with an incidence of approximately 0.3-0.5% throughout world. The characteristic event is seizure which is paradoxical event due to abnormal, excessive, hyper synchronous discharges from an aggregate in CNS.

Epilepsy is characterized by recurrent seizures. Pathophysiology of epilepsy involves alternation in voltage dependent ion channels.

(a) Reduction in inhibitors i.e., GABA - mediated

(b) Increase in excitatory i.e., Glutamate - mediated inputs.

Anti epileptic drugs act by modularly GABA or Glutamate transmission or by modularly sodium and calcium ion channels.

A. In vitro Methods

(i) Hippocampal slices
(ii) In vitro assay for GABAergic compounds
(iii) Excitatory amino acid receptor binding assay
(iv) Electrical recording from isolated brain cells

B. In vivo Methods

(i) Electrically induced seizures
(ii) Other methods of kindling
(iii) Chemically induced convulsions
(iv) Seizures induced by focal lesions
(v) Models of status epileptics
(vi) Models of infantile spasm
(vii) Genetic animal models of epilepsy

Preclinical screening of **antiepileptic drugs (AEDs)** helps to test their **effectiveness** and **safety** before human trials. These drugs are used to treat **epilepsy** and prevent **seizures**.

✅ **In Vivo Models (Animal-Based Tests)**

1. Maximal Electroshock Seizure (MES) Test

Aim:
To evaluate if the drug can **prevent or reduce seizures** induced by an **electrical shock**.

Procedure:

- A **mild electrical shock** is applied to the animal (usually a rat or mouse) to induce a **seizure**.
- The test drug is administered before the shock.
- **Observe** the animal's seizure activity:
  - **Duration and severity of seizures**
  - **Type of seizure activity** (clonic, tonic, etc.)

Conclusion:
If the drug **reduces seizure duration** or prevents seizures altogether, it may be an **effective antiepileptic drug**.

2. Kindling Model (Repeated Stimulation-Induced Seizures)

Aim:
To test the drug's ability to **prevent seizure development** over time, simulating chronic epilepsy.

Procedure:

- **Low electrical stimulation** is given to the animal's brain over several days.
- Each stimulation causes **seizures** that **increase in severity** over time (kindling effect).
- The test drug is administered and its effect on seizure **development and progression** is observed

◈ **Conclusion:**
If the drug **prevents the escalation of seizures**, it could be a promising treatment for **chronic epilepsy**.

✅ **In Vitro Models (Cell-Based Tests)**

□ **1. Patch-Clamp Technique (Ion Channel Activity)**

◈ **Aim:**
To study how the drug affects **ion channels** that play a key role in **seizure generation**.

◈ **Procedure:**

- **Cultured neurons** are used in a **petri dish**.
- The neurons are exposed to the drug.
- The **patch-clamp technique** measures the electrical activity of individual **ion channels** (like sodium, potassium, or calcium channels) involved in **neuron firing**.
- Changes in ion flow and neuronal firing patterns are recorded.

◈ **Conclusion:**
If the drug **reduces excessive ion channel activity**, it may be effective in **controlling seizures** by stabilizing neuronal excitability.

□ **2. In Vitro Seizure Induction in Hippocampal Slices**

◈ **Aim:**
To test the drug's ability to **prevent seizures** in a controlled brain slice model, especially for **temporal lobe epilepsy**.

◈ **Procedure:**

- **Brain slices** (usually from the hippocampus) are placed in a **dish** and **stimulated electrically** to cause seizure-like activity.
- The test drug is added to the medium.
- **Seizure-like activity** (increased firing of neurons) is monitored using **electrodes** or **voltage-sensitive dyes**.

◈ **Conclusion:**
If the drug **reduces seizure-like activity** in these brain slices, it shows potential for controlling **epileptic activity**.

📌 **Conclusion:**

- **In vivo models** simulate real-life seizure conditions and help assess **seizure prevention** and **long-term effects**.
- **In vitro models** focus on understanding **cellular mechanisms** and the **ion channel** activity involved in seizures.

These preclinical tests are essential to determine if a drug is safe and effective enough for **human trials**.

Screening Methods for Drugs Influencing Learning and Memory (NOOTROPICS)

✓ 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. 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 main features of nootropic drugs 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 cortical sub cortical control mechanisms.

❖ *Screening methods for drugs used to enhancing memory and intelligence*

In vivo models:

(a) Morris water maze test
(b) Assessment of learning memory using Y maze apparatus
(c) Passive shock avoidance paradigm

(d) Assessment of learning and memory using Hebb's William Maze (rectangular maze)
(e) Scopolamine induced amnesia (Interceptive Behavior model)
(f) Elevated plus maze (Exteroceptive behavior model)
(g) Shuttle box avoidance (Two way shuttle box)
(h) Passive avoidance paradigm (Exteroceptive behavior model)

In vitro models:

(a) In vitro inhibition of acetylcholine-esterase activity in rat striatum
(b) Ex vivo cholinesterase inhibition
(c) [H]-N-methyl scopolamine binding in the presence and absence of GPP(NH)p
(d) [3H]N-methylcarbamylcholine binding to nicotinic cholinergic receptors in rat frontal cortex.
(e) Cultured neurons/astroglial cells

✅ In Vivo Models (Tests on Live Animals)

These models use animals like **mice or rats** to study how a drug works **inside the body**.

🐁 1. Morris Water Maze Test

◆ **Aim:**

To test if a drug helps improve **learning and memory** in animals.

◆ **Procedure:**

- A large round tank is filled with water and has a **hidden platform** under the surface.

- The animal (mouse or rat) is placed in the water at different points.
- It tries to **find the platform** to escape the water.
- The test drug is given before or during the learning period.
- **Time taken** to find the platform is recorded over several days.

◆ **Conclusion:**

If the animal **learns faster** and finds the platform **more quickly each day**, the drug may be improving memory.

Faster learning = Nootropic effect

🐁 2. Elevated Plus Maze (Transfer Latency Test)

◆ **Aim:**

To check if the drug helps with **learning and memory recall**.

◆ **Procedure:**

- The maze has **2 open arms** and **2 closed arms** in a plus (+) shape, placed above ground.
- On **Day 1**, place the animal in an **open arm** and time how long it takes to move to a **closed arm**. This is called **transfer latency**.
- On **Day 2**, repeat the same steps.
- The test drug is given before Day 1 or Day 2.

◆ **Conclusion:**

If the animal **takes less time on Day 2**, it means it **remembered** the closed arm is safer.

Shorter time = Improved memory

In Vitro Models (Lab Tests in Dishes or Tubes)

These models use **cells or tissues in lab dishes** to test how a drug works at the cellular or molecular level.

1. Acetylcholinesterase (AChE) Inhibition Assay

◆ **Aim:**
To check if the drug helps increase **acetylcholine** — a chemical important for memory.

◆ **Procedure:**

- Acetylcholine is a brain chemical needed for **learning and focus**.
- An enzyme called **acetylcholinesterase (AChE)** breaks it down.
- In this test, the drug is added to a solution containing **AChE enzyme**.
- If the drug **blocks** the enzyme, more acetylcholine remains.

◆ **Conclusion:**
If the drug **inhibits AChE**, it keeps more acetylcholine active in the brain.
✅ **More acetylcholine = Better memory = Nootropic action**

2. Neuronal Cell Viability Assay (MTT Assay)

◆ **Aim:**
To test if the drug **protects brain cells** or helps them **survive and grow**.

◆ **Procedure:**

- Brain cells are grown in a dish (neurons or similar cells).
- The drug is added to see if it helps **cells survive**, especially under **stressful conditions** (like oxidative stress).

- A chemical dye (MTT) is added, which changes color if the cells are alive.
- The **darker the color**, the **more living cells**.

◆ **Conclusion:**

If the cells **survive better** with the drug, it may protect the brain and help **improve memory and learning**.

✅ **More live cells = Neuroprotective = Nootropic effect**

Screening Methods for Anti Parkinsonism Activity

- More than 2.1 million people worldwide suffer from Parkinson's disease (PD)
- A neurological syndrome characterized by bradykinesia, postural instability, rigidity and involuntary tremors. Extensive loss of dopaminergic neurons of substantia nigra.
- Biochemically there is depletion of dopamine increases of Acetylcholine (Ach).
- Neurotoxicity in CNS and basal ganglia and produces neurological symptoms.
- Belladonna alkaloids, antispasmodics and antihistamics, are traditionally used for management of Parkinsonism. Current drug therapy with levodopa which is decarboxylated to dopamine in dopaminergic neurons which helps to maintain adequate motor functions.

DEPTH OF BIOLOGY

Parkinson's Disease (PD): Overview

Parkinson's disease is a brain disorder that mainly affects **movement**.
It is caused by a **loss of dopamine-producing neurons** in a part of the brain called the **substantia nigra**.

Symptoms include:

- Tremors
- Muscle stiffness
- Slow movements
- Balance problems

Drugs for PD aim to:

- **Increase dopamine levels**
- **Protect dopamine neurons**
- **Improve motor function**

✅ In Vivo Models (Animal-Based Tests)

These models use **live animals** (usually rats or mice) to study motor symptoms and drug effects.

🐀 1. Haloperidol-Induced Catalepsy Model

◆ Aim:
To test **anti-Parkinson activity** by measuring a drug's ability to **reduce catalepsy** (muscle stiffness and immobility).

◆ Procedure:

- Inject the animal (usually a rat) with **haloperidol**, a drug that blocks dopamine and causes **catalepsy** (Parkinson-like symptoms).
- Give the **test drug**.
- Place the rat's front paws on a horizontal bar.
- Measure how long it stays in that position (immobile).

◆ **Conclusion:**
If the test drug **reduces the time of immobility**, it may have **anti-Parkinson activity** by restoring dopamine function.

✅ **Less stiffness = Dopamine boost = Anti-Parkinson effect**

🧠 2. 6-OHDA (6-Hydroxydopamine) Lesion Model

◆ **Aim:**
To create a model of **Parkinson's disease** by selectively destroying dopamine neurons.

◆ **Procedure:**

- Inject **6-OHDA** into one side of the rat's brain (substantia nigra or medial forebrain bundle).
- This causes loss of dopamine neurons on that side.
- Inject **apomorphine**, which causes the animal to **rotate in circles** due to imbalance.
- Test drugs are given to see if they reduce the number of rotations or restore balance.

◆ **Conclusion:**
If the drug **reduces turning behavior** or protects neurons, it may help in treating Parkinson's.

✅ **Fewer rotations = Neuroprotection or dopamine recovery**

✅ In Vitro Models (Lab/Cell-Based Tests)

These models use **brain cells or tissue** in the lab to test cellular-level effects.

🧪 1. Dopamine Receptor Binding Assay

🔹 Aim:
To test whether a drug **binds to dopamine receptors** (especially D1 or D2), which are important in Parkinson's treatment.

🔹 Procedure:

- Use brain tissue or cells with dopamine receptors.
- Add a **radioactive or fluorescent marker** that normally binds to dopamine receptors.
- Add the **test drug** to see if it displaces the marker.

🔹 Conclusion:
If the test drug **binds well to dopamine receptors**, it may help replace lost dopamine function in Parkinson's.

✅ **Strong receptor binding = Potential anti-Parkinson drug**

🧪 2. Cell Viability Test Using Neurotoxin (e.g., MPP^+ Model)

🔹 Aim:
To test if a drug can **protect dopamine-producing cells** from damage.

🔹 Procedure:

- Grow **dopaminergic neurons** in a lab dish.

- Add a neurotoxin like **MPP⁺** to damage the cells (mimics Parkinson's).
- Then add the **test drug**.
- Use MTT or other dye-based assays to check how many cells **survive**.

◆ **Conclusion:**
If more cells **stay alive** with the drug, it may have **neuroprotective** effects helpful in Parkinson's disease.

✅ **More surviving cells = Brain protection = Anti-Parkinson potential**

Final Summary (in Simple Words):

- **In vivo tests** show how well the drug works in living animals (motor skills, stiffness, brain damage).
- **In vitro tests** show how the drug interacts with brain cells or receptors in the lab.
- If the drug **restores dopamine activity**, **protects brain cells**, or **improves movement**, it may be useful for **Parkinson's disease**.

What Is Alzheimer's Disease (AD)?

Alzheimer's is a **progressive brain disorder** that causes **memory loss**, **confusion**, and **thinking difficulties**, especially in older adults. It is mainly caused by:

- **Loss of cholinergic neurons**

- **Accumulation of amyloid-beta plaques**
- **Tau protein tangles**

Symptoms of Alzheimer's Disease

1. **Memory loss** – especially forgetting recent events or names.
2. **Confusion** – about time, place, or people.
3. **Difficulty speaking** – trouble finding words or following conversations.
4. **Trouble with daily tasks** – like cooking, driving, or handling money.
5. **Misplacing things** – putting items in strange places and not remembering wher

Drugs for AD aim to:

- Improve **memory**
- Increase **acetylcholine** levels
- Protect brain cells

✅ **In Vivo Models (Animal-Based Tests)**

1. Scopolamine-Induced Memory Impairment Test

◆ **Aim:**
To check whether the drug can **reverse memory loss** caused by scopolamine.

◆ **Procedure:**

- Scopolamine is given to animals (rats/mice) to block acetylcholine and cause **temporary memory loss**.

- A memory test (e.g., Morris Water Maze or Y-Maze) is performed after giving the **test drug**.
- Measure the animal's ability to remember the task.

◆ **Conclusion:**
If the test drug **improves memory performance** (e.g., finds the platform faster or makes correct choices), it may be helpful in **treating Alzheimer's**.

✅ Better performance = Memory improvement = Anti-Alzheimer's effect

🐁 **2. Transgenic Mouse Model (APP/PS1 or 3xTg-AD Mice)**

◆ **Aim:**
To study the drug's effect in a mouse that **naturally develops Alzheimer-like symptoms**.

◆ **Procedure:**

- These genetically modified mice develop **amyloid plaques** and **memory loss** as they age.
- The **test drug** is given over weeks/months.
- Behavioral memory tests are done (e.g., Novel Object Recognition).
- Brain tissues are examined for **plaque buildup**.

◆ **Conclusion:**
If the drug **improves memory** and **reduces plaques or inflammation**, it may be effective for **Alzheimer's treatment**.

✅ ↓ Amyloid plaques + ↑ Memory = Positive effect in AD

In Vitro Models (Lab/Cell-Based Tests)

1. Acetylcholinesterase (AChE) Inhibition Assay

◆ **Aim:**

To test whether the drug **blocks the enzyme** that breaks down **acetylcholine** (important for memory).

◆ **Procedure:**

- Mix acetylcholine and AChE enzyme with the test drug in a tube.
- Measure enzyme activity using a color reaction (Ellman's method).
- Less color = more inhibition.

◆ **Conclusion:**

If the drug **inhibits AChE**, it helps maintain higher levels of acetylcholine in the brain, which is beneficial in AD.

✅ AChE inhibition = ↑ Acetylcholine = Better memory

2. Amyloid-Beta Toxicity Assay in Neuronal Cells

◆ **Aim:**

To test if the drug can **protect brain cells** from damage caused by **amyloid-beta**, a toxic protein in AD.

◆ **Procedure:**

- Cultured brain cells (neurons) are exposed to **amyloid-beta**, which usually kills them.
- The test drug is added to see if it can **prevent cell death**.
- Use an MTT or LDH assay to measure how many cells survive.

◆ **Conclusion:**
If more cells **stay alive**, the drug shows **neuroprotective effects** and may help in **treating Alzheimer's**.

✅ ↑ Cell survival = Brain protection = Anti-Alzheimer's activity

Summary

- **In vivo tests** show if the drug helps animals with **memory loss** or **brain damage**.
- **In vitro tests** show if the drug can **protect brain cells** or **boost brain chemicals**.
- A good anti-Alzheimer's drug will usually:
  - Improve memory
  - Increase acetylcholine
  - Protect neurons
  - Reduce amyloid plaques

□ What is Multiple Sclerosis? (MS)

Multiple Sclerosis (MS) is a **disease of the brain and spinal cord** (central nervous system) where the **immune system attacks the protective covering of the nerves** called the **myelin sheath**. This causes problems in how messages travel between the brain and the body.

⚠ Symptoms of MS (in simple language):

1. **Weakness in arms or legs**

2. **Tingling or numbness** (like pins and needles)
3. **Vision problems** (blurry or double vision)
4. **Balance and coordination issues**
5. **Tiredness (fatigue)**
6. **Muscle stiffness or spasms**
7. **Problems with memory or thinking**
8. **Difficulty walking**

✅ **In Vivo Models (Animal Tests)**

1. Experimental Autoimmune Encephalomyelitis (EAE) Model

◆ **Aim:**

To check if a drug can **reduce inflammation** and **damage** in the brain and spinal cord, similar to what happens in MS.

◆ **Procedure:**

1. **EAE Induction**:
    - Animals (usually mice or rats) are injected with a substance that makes their immune system attack their own **myelin**, which is similar to MS.
2. **Drug Treatment**:
    - After the animals get the disease, the drug being tested is given to them.
3. **Monitoring**:
    - Scientists look at how the animals' **motor skills** (walking, moving) are affected.
4. **Tissue Check**:

- After the test, the brain and spinal cord are checked for **damage**.

◆ **Conclusion:**

- If the drug **helps reduce damage** and improves **movement**, it may be useful for **treating MS**.

2. Theiler's Murine Encephalomyelitis Virus (TMEV) Model

◆ **Aim:**

To see if the drug can stop **progressive damage** to the brain and nerves caused by a **virus**, which is similar to **MS**.

◆ **Procedure:**

1. **Virus Injection**:
   - Mice are infected with a virus that causes **nerve damage**, similar to what happens in MS.
2. **Drug Treatment**:
   - After infection, the drug is given to see if it **stops** or **slows down** the damage.
3. **Observation**:
   - The animals are observed for **weakness** or **problems moving**, and scientists check for **brain damage** after the test.

◆ **Conclusion:**

- If the drug **reduces damage** and **improves movement**, it may be a good treatment for **MS**.

✅ **In Vitro Models (Cell Tests)**

1. Oligodendrocyte Culture Model

◆ **Aim:**

To test if a drug can help **repair myelin** and **protect brain cells** that make myelin, which is important in MS.

◆ **Procedure:**

1. **Growing Cells**:
    - Scientists grow brain cells called **oligodendrocytes** in a dish.
2. **Drug Treatment**:
    - The drug is added to see if it helps these cells **make myelin** or **survive better**.
3. **Analysis**:
    - After treatment, scientists check if the cells **survived** and whether they **produced myelin**.

◆ **Conclusion:**

- If the drug **helps cells survive** and **makes more myelin**, it could be helpful for **MS treatment**.

2. Amyloid Beta Toxicity Model

◆ **Aim:**

To see if a drug can protect **brain cells** from damage caused by a protein called **amyloid beta**, which can harm neurons in MS.

◆ **Procedure:**

1. **Cell Culture**:
    - Brain cells are grown in a dish.
2. **Amyloid Beta**:

- Amyloid beta is added to the cells to **cause damage**.

3. **Drug Treatment**:
   - The drug is tested to see if it **protects the cells** from this damage.
4. **Analysis**:
   - Scientists check if the drug helps **more cells survive** after the damage.

◆ **Conclusion:**

- If the drug **protects cells** and **helps them survive**, it could be used for **MS treatment**.

📋 **Summary:**

- **In vivo tests** (on animals) show how the drug affects **brain and nerve damage** and **movement** in MS-like conditions.
- **In vitro tests** (in lab dishes) test whether the drug can **protect brain cells** and **help repair myelin**.

These tests help figure out if a drug could be **useful for MS treatment** before human trials.

Preclinical Screening of Drugs Acting on the Autonomic Nervous System (ANS)

The **autonomic nervous system (ANS)** regulates involuntary physiological functions such as heart rate, blood pressure, digestion, and respiration. It is divided into two branches:

1. **Sympathetic Nervous System (SNS)** – responsible for "fight or flight" responses.
2. **Parasympathetic Nervous System (PNS)** – responsible for "rest and digest" responses.

Drugs acting on the **ANS** can either **enhance** (agonists) or **inhibit** (antagonists) the functions of the SNS or PNS. The preclinical screening of these drugs is essential for evaluating **efficacy, safety**, and **mechanism of action** before advancing to clinical trials.

This screening typically involves **in vitro**, **in vivo**, and **animal alternative models** to study how these drugs affect **neurotransmission**, **receptor activity**, and **physiological responses**.

- **Sympathomimetics** are drugs that **mimic the effects** of **sympathetic nervous system stimulation**, typically by acting on **adrenergic receptors** (like alpha and beta receptors). These drugs can **increase heart rate**, **blood pressure**, and other functions. They are often used for conditions like asthma, shock, and nasal congestion.

✅ **In Vivo Test (Animal Model)**

Test Name: The Isolated Heart Preparation (Langendorff Method)

◆ **Aim:**

To assess the **cardiovascular effects** (heart rate, contractility, and blood pressure) of a **sympathomimetic drug** in a controlled, isolated environment.

◆ **Procedure:**

1. **Preparation of the Heart**:
    - In this test, the **heart** of an animal (usually a rat or rabbit) is removed and placed in a special solution (called a **buffer solution**) that keeps it alive.
2. **Drug Administration**:
    - The sympathomimetic drug is administered either by direct **injection** or through the solution the heart is placed in.
3. **Monitoring**:
    - The **heart rate**, **force of contraction**, and **blood flow** are measured.
    - The effects of the drug are observed to see if it causes an **increase in heart rate** or **force of contraction**, similar to what happens when adrenaline is released.
4. **Data Collection**:
    - Parameters such as **rate of contraction**, **blood pressure**, and **cardiac output** are recorded.

◆ **Conclusion:**

- If the drug **increases heart rate** or **force of contraction**, it is **stimulating the sympathetic nervous system**, and therefore, it is a **sympathomimetic**.
- This test helps determine the **strength** and **duration** of the drug's **cardiovascular effects**.

✅ **In Vitro Test (Cell Culture)**

Test Name: Beta-Adrenergic Receptor Activation Test

◆ **Aim:**

To test if a **sympathomimetic drug** can activate **beta-adrenergic receptors** in cultured cells, causing the **increase in cellular activity** (such as cAMP production, which is a common pathway for sympathomimetic action).

◆ **Procedure:**

1. **Culturing Cells**:
    - **Beta-adrenergic receptor-expressing cells** (often **CHO cells**, a type of mammalian cell) are cultured in a dish.
2. **Drug Administration**:
    - The sympathomimetic drug is added to the cells.
3. **Monitoring cAMP Production**:
    - When **beta-adrenergic receptors** are activated by the drug, they trigger the production of **cAMP** (cyclic adenosine monophosphate), which is a secondary messenger that increases cellular activity.
    - cAMP production is measured using special assays.
4. **Data Collection**:
    - The amount of **cAMP** in the cells is measured, typically using a **cAMP assay**.
    - The **increase in cAMP** indicates the drug is activating **beta-adrenergic receptors**.

Parasympathomimetics are drugs that mimic the effects of the parasympathetic nervous system, typically by activating muscarinic receptors. These drugs can cause effects like

slowing down the heart rate, increasing secretions (like saliva), and promoting digestion. They are often used for conditions like glaucoma, myasthenia gravis, and urinary retention.

✅ **In Vivo Test (Animal Model)**

Test Name: The Effect on Heart Rate (Bradycardia Test)

◆ **Aim:**

To test if the drug can **lower the heart rate** by stimulating the **parasympathetic nervous system**, which generally causes the heart to slow down (called **bradycardia**).

◆ **Procedure:**

1. **Animal Preparation**:
   - An animal (commonly a **rat** or **rabbit**) is used for the test. The animal is placed in a **controlled environment** where its heart rate can be monitored.
2. **Drug Administration**:
   - The **parasympathomimetic drug** is given to the animal, typically **via injection** or **oral administration**.
3. **Heart Rate Monitoring**:
   - The heart rate is measured before and after drug administration. This can be done using **ECG (electrocardiogram)** or by directly monitoring the heart rate.
4. **Observation of Effect**:
   - After the drug is administered, scientists observe whether the drug causes the heart rate to **slow down**.

◆ **Conclusion:**

- If the drug **slows down the heart rate**, it shows that it is activating the **parasympathetic nervous system**, which is a typical effect of **parasympathomimetics**.
- A **decreased heart rate** (bradycardia) suggests the drug is acting as a **parasympathomimetic**.

✅ **In Vitro Test (Cell Culture)**

Test Name: Muscarinic Receptor Activation Assay

🔹 **Aim:**

To test if the **parasympathomimetic drug** can activate **muscarinic receptors** on cells, leading to **cellular responses** like increased **salivation** or **smooth muscle contraction**.

🔹 **Procedure:**

1. **Cell Culture**:
    - **Muscarinic receptor-expressing cells** (often **CHO cells** or **smooth muscle cells**) are grown in a **dish** in a controlled lab environment.
2. **Drug Administration**:
    - The **parasympathomimetic drug** is added to the cultured cells.
3. **Monitoring Cellular Response**:
    - The drug's effect is measured by testing for changes like an increase in **intracellular calcium levels**, **smooth muscle contraction**, or changes in **electrical activity** in the cells (which are responses to muscarinic receptor activation).
4. **Data Collection**:

- These cellular responses are measured using **fluorescent assays** or **muscle contraction assays**

In Simple Terms:

- **In Vivo Test**: **Heart rate test** checks if the drug **slows down the heart**, which shows it's acting like the **parasympathetic nervous system**.
- **In Vitro Test**: **Cell culture test** sees if the drug **activates muscarinic receptors** on cells, causing them to **react** (like contracting muscles or changing electrical activity).

  These tests help determine if a drug is a **parasympathomimetic**, meaning it **mimics** the effects of the **parasympathetic nervous system**, usually causing **slower heart rate** and other calming effects.