Pharmacological Screening of Anti Psychotic Agents

(Pharmacological and Toxicological Screening Methods)

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- Screening models

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Introduction

- Psychosis refers to losing touch with reality.
- These are severe psychiatric illness with serious distortion of thought, behavior, capacity to recognize reality and of perception (delusions and hallucinations).
- There is inexplicable misperception and misevaluation; the patient is unable to meet the ordinary demands of life.

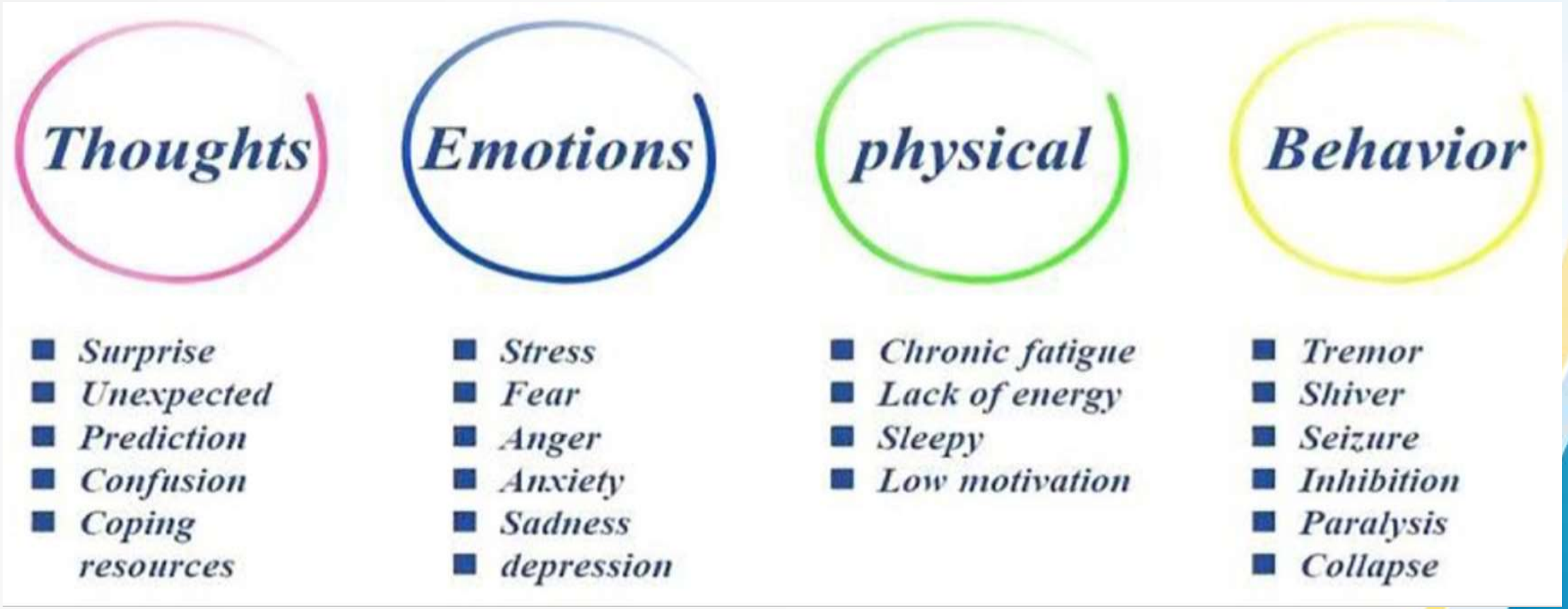
Acute and chronic organic brain syndromes (cognitive disorders):

Delirium and dementia.

Cases: Exposure to toxic substances or pathological changes.

Prominent features are confusion, disorientation, defective memory and disorganized behavior.

Functional disorders:



Psychosis can be broadly categorized in to four groups

1.**Acute and Chronic organic brain syndrome (cognitive disorders):**

- Such as, delirium and demantia, prominent features of confusion, disorentation, defective memory and disorganized behaviour.

2. **Functional disorders:** such as, Memory and orientation mostly retained by emotion, thought, reasoning and behaviour are altered.

3. **Schizophernia (split mind):** i.e.splitting of perception and interpretation from reality- hallucination, inability to think coherently.

- Schizophernia is often described in the terms of positive and negative symptoms.

4. **Paranoid state:** i.e. fixed delusions (false beliefs) and loss of insight in to abnormality.

Schizophrenia:

- Schizophrenia is a particular type of psychosis (that is, a mental disorder caused by some inherent dysfunction of the brain).
- It is characterized by delusions, hallucinations (often in the form of voices), and thinking or speech disturbances.
- This mental disorder is a common affliction, occurring in about 1 percent of the population.

Paranoid states:

- An unrealistic distrust of others or a feeling of being persecuted. Extreme degrees may be a sign of mental illness.

Mood (affective) disorders:

- **Mania:** The elation or irritable mood, reduced sleep, hyperactivity, uncontrollable thought and speech, may be associated with reckless or violent behavior.
- **Depression:** The sadness, loss of interest and pleasure, worthlessness, guilt,

physical and mental slowing, melancholia,

Bipolar Disorder:

- Bipolar disorder causes extreme mood swings that include emotional highs (mania or hypomania) and lows (depression).

Antipsychotic Drugs:

- Antipsychotic drugs, often referred to as neuroleptics or major tranquilizers, are used primarily to treat schizophrenia, but they are also effective in other psychotic states, including manic states with psychotic symptoms such as paranoia, and hallucinations, and delusions.

- Antipsychotic drugs are not curative and do n eliminate the chronic thought disorder, but the decrease the intensity of hallucinations and delusions and permit the person with schizop to function in a supportive environment.

Pharmacological Classification:

A. First-generation Antipsychotics:

1. Low Potency:

- Chlorpromazine
- Prochlorperazine
- Thioridazine

2. High potency:

- Fluphenazine
- Haloperidol
- Pimozide
- Thiothixene

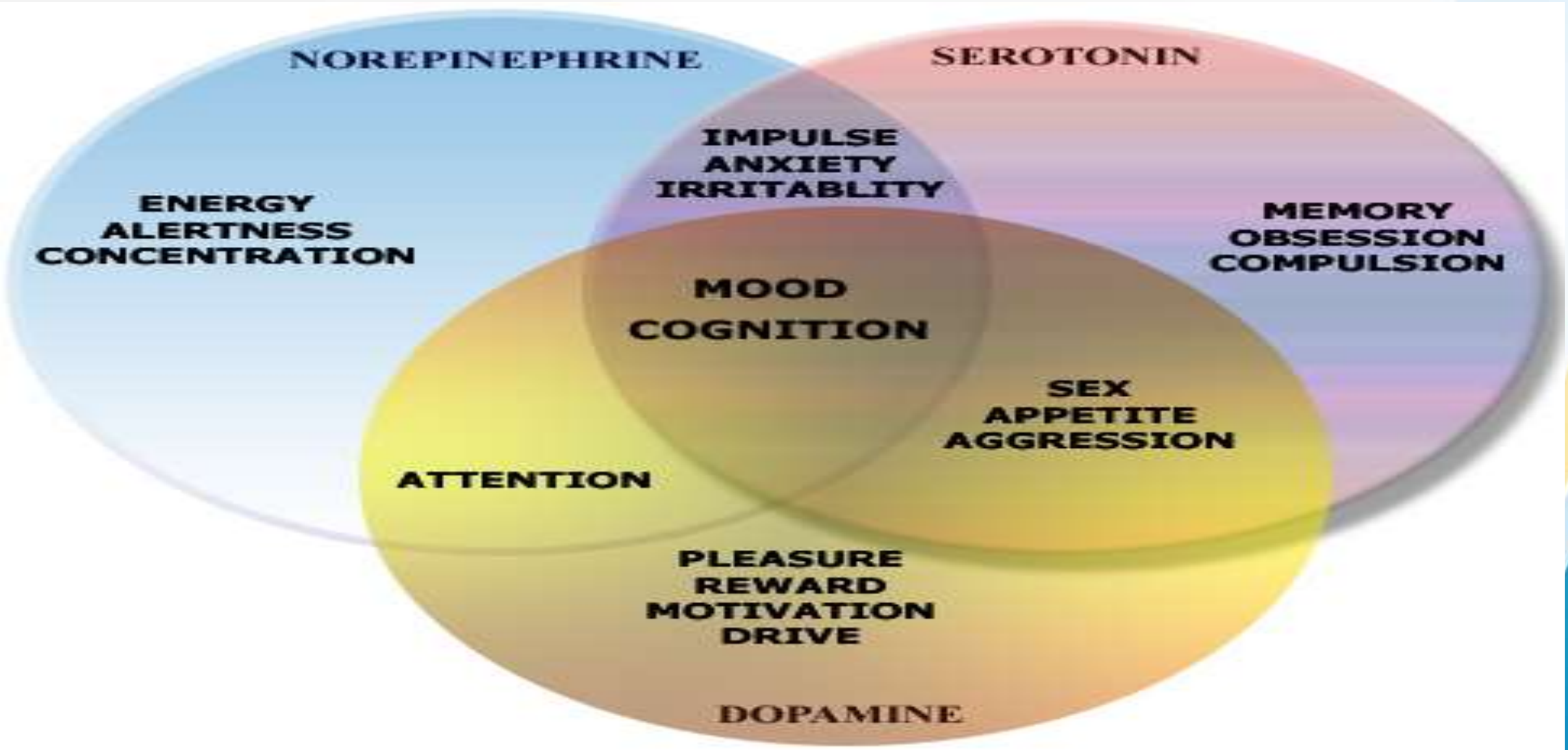
B. Second-generation Antipsychotics:

Aripiprazole

Asenapine

Clozapine

Risperidone


NOREPINEPHRINE
SEROTONIN
IMPULSE
ANXIETY
IRRITABLITY
ENERGY
ALERTNESS
CONCENTRATION
MEMORY
OBSESSION
COMPULSION
MOOD
COGNITION
SEX
APPETITE
AGGRESSION
ATTENTION
PLEASURE
REWARD
MOTIVATION
DRIVE
DOPAMINE

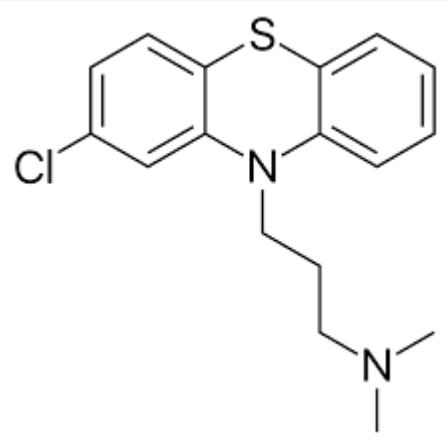
A. First-generation Antipsychotics:

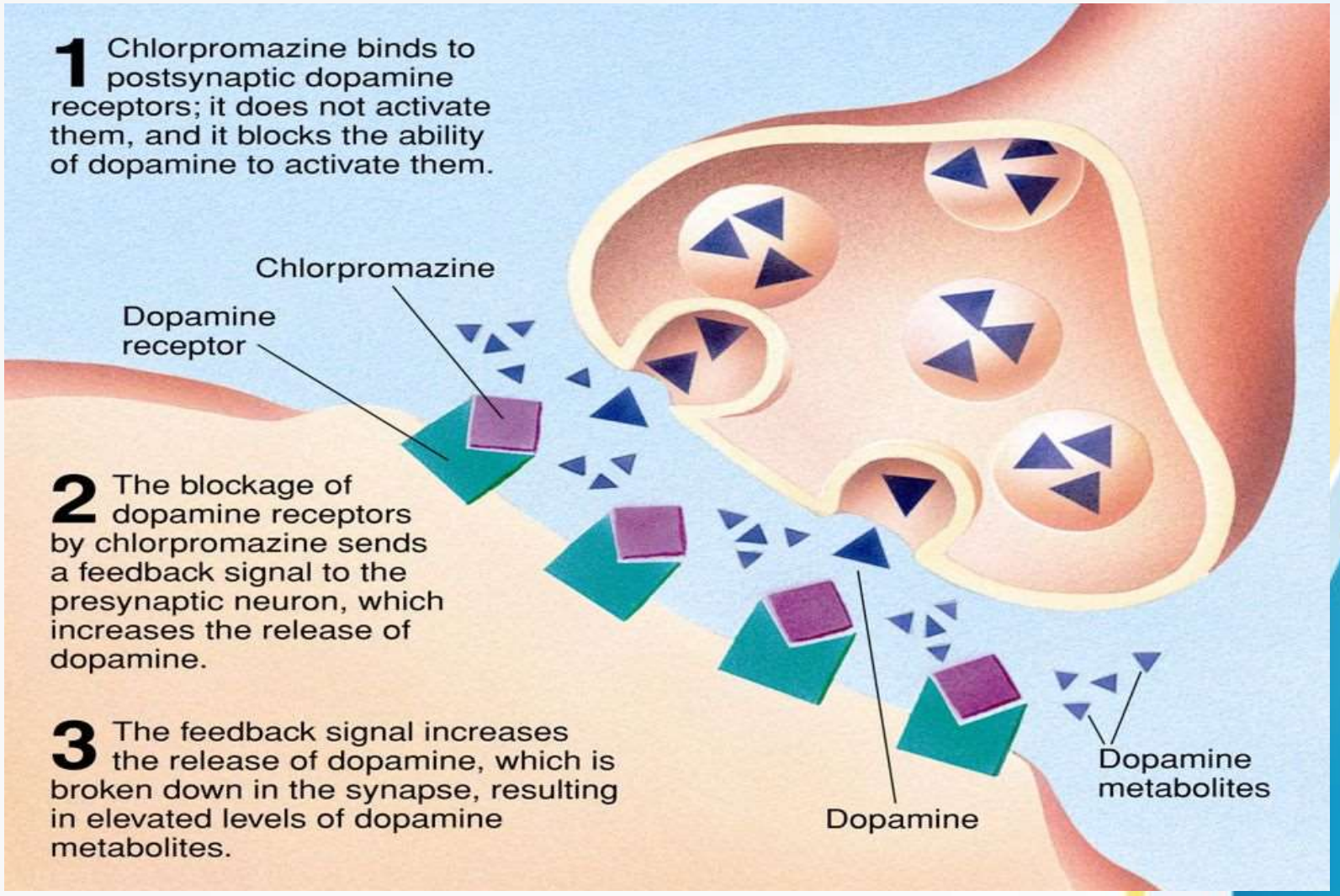
- The first-generation Antipsychotic drugs (also called conventional, typical, or traditional antipsychotics) are **competitive inhibitors** at a variety of receptors, but their antipsychotic effects reflect competitive **blocking** of **D2 dopamine receptors**.

B. Second-generation Antipsychotics:

- The second generation Antipsychotic drugs (also referred to as “atypical” antipsychotics) have fewer extrapyramidal symptoms (EPS) than the first-generation agents, but are associated with a higher risk of metabolic side effects, such as diabetes, hypercholesterolemia, and weight gain.
- The second-generation drugs appear to owe their unique activity to **blockade** of both **serotonin** and **dopamine receptors**.

Pharmacology of chlorpromazine (CPZ):





Screening Models

IN-VIVO MODELS

- **Behavioral Tests**

1. Catelepsy in rodents.
2. Golden hamsters test.
3. Pole climb avoidance in rats.
4. Conditioned avoidance reflex in rats.

- **Test Based on Pharmacologic antagonism**

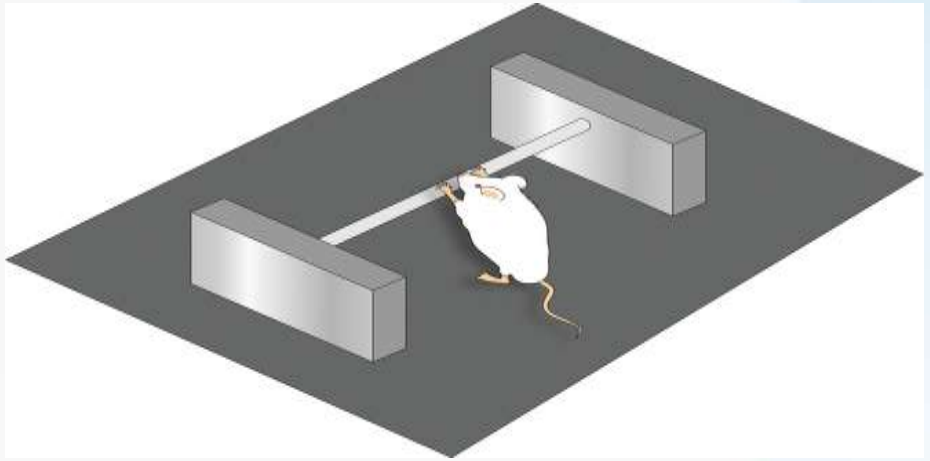
1. Inhibition of Amphetamine-induced stereotype in rats.
2. Inhibition of Apomorphine -induced stereotype in mice.
3. Neuro Developmental model.

IN VITRO MODELS

1. D1- receptor assay
2. D2- receptor assay
3. Binding to D3- receptor
4. Binding to D4- receptor
5. α1-Adrenergic receptors binding in brain

IN VIVO MODELS

- **Catelepsy in Rodents**



1. Purpose and Rationale

Catalepsy in rats is defined as a failure to correct an externally imposed, unusual posture over a prolonged period of time. This is a typical effect of all agents which inhibits dopaminergic system in nigrostratum.

PROCEDURE

- Groupings→ group I - treated with test drug

  Group II - treated with standard drug

  (haloperidol 0.2mg/kg I.P)

- Animals are placed inside the translucent plastic boxes.

↓

- After some time each animal is grasped gently around the shoulders and under the forepaws and placed carefully on the dowel.

↓

- The amount of time spent atleast with one forepaw on the bar is determined.

- When the animal removes its paws, the time is recorded and the rat is repositioned on the bar.

↓

- Four trails are conducted for each animal at 30, 60, 120 & 360 min. An animal is considered cataleptic if it remains on the bar for 60 sec.

Evaluation

- The percentage of cataleptic animal is calculated for each group. ED_{50} valued are calculated for comparison & elucidition of potency.

- **Inhibition of Amphetamine induced Stereotype in Rats**

1.Purpose & Rationale

- Amphetamine is an indirect acting ssympathomimetic agent which releases catacholamines from its neurol storage pools.
- In rats the drug induces characteristic stereotypic behaviour and this can be prevented by neuroleptics.

PROCEDURE

- Groupings;

  Group 1 (control) - d-Amphetamine 10 mg/kg S.C.

  Group II (treated) - Chlorpromazine 10 mg/kg S.C

  d-Amphetamine 10 mg/kg S.C.

- The rats with a body weight between 120- 200g are selected and grouped into 6 each in control and treated.

↓

- They are injected simultaneously with d- Amphetamine and test compound and placed in individuals cages.

↓

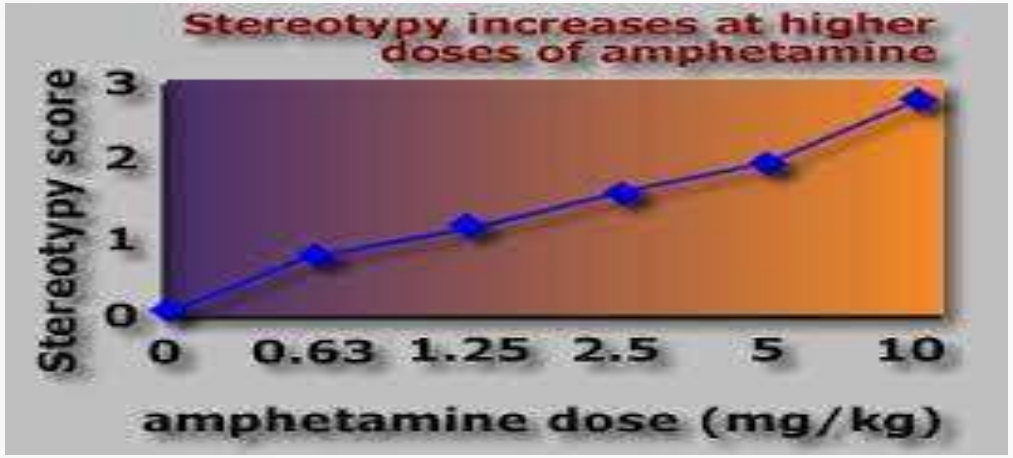
- After 60 min of drug administeration the rats are observed.

↓

- In the group I animals which have been administred d- Amphetamine

Evaluation

- The % effectiveness of a drug is determined by the number of animals protected in each group.
- If the group of animals which treated with test drug is protected by stereotypic behaviour, the efficacy of a drug is confirmed as neuroleptic agent.



- **Golden Hamster Test**

1.Purpose & Rationale

- This test uses the innate behavior of the species (Mesocricetus auratus) for differentiation between neuroleptics & sedative-hypnotic activity. The aggressive behaviour of male Golden hamster is suppressed by neuroleptic in doses which do not impair motor function.

PROCEDURE

- Control vehicle treated (no.6)

- Treated standard drug (no.6)

- The above grouped animals are placed together in crowded conditions in specially designed cages for at least 14 days.

↓

- The animals develop a characteristic fighting behavior during this period.

For Selection of a test Group

- From the above, single animals are placed into a glass jars of 2 lit.

↓

- Animals assume a squatting resting position during day.

↓

- If animals are touched with a stick or forceps, they wake up from their day time sleep & arouse immediately from resting position (a characteristic behavior is seen).

↓

- Tries to hold the hamster with a blunted forceps.

↓

- The hamster throws himself onto his back & tries to bite & to push the forceps away with his legs & utters angry shrieks.

↓

- Touching the animal is repeated up to 6 times followed by punching with forceps.

↓

- Only the animals responding to stimulus with all defensive reactions (turning, vocalizing, biting) are included for the test group.

↓

- The test drug is given by S.C. route. Six animals are used for each dose.

↓

- Observe the suppression of aggressive behavior of Golden hamsters in doses

Evaluation

> The stimuli are applied every 20 min for 3 hr.

> The suppression of defense reaction is evaluated.

> If all defense reactions are suppressed even after punching with forceps at least once during test period, an animal is regarded to be completely 'tamed".

IN VITRO MODELS

D_2 Receptor Assay: H-Spiroperidol Binding

1.Purpose & Rationale

- The neuroleptic compound Haloperidol has been used as binding ligand to study the activity of other neuroleptics.
- The use of Haloperidol has been superseded by Spiroperidol.
- Dopamine receptor binding assays employing dopaminergic antagonists in mammalian striatal tissue, a dopamine enriched area of the brain, have been shown to be predictive of in vivo dopamine receptor antagonism and antipsychotic activity.
- Spiroperidol is considered to be an antagonist specific for D_2 receptors.

PROCEDURE

➢ Tissue Preparation

- Male Wistar Rats are decapitated, their corpora striate removed, weighed and homogenized in 50 ml of ice-cold 0.05 M Tris buffer (pH7.7) .
- The homogenate is centrifuged at 400 rpm for 15 min.
- The pellet is rehomogenized in fresh buffer and recentifuged at 400 rpm.
- The final pellet is then resuspended in Tris buffer.

ASSAY

- The membrane preparations are incubated with H- Spiroperidol.

↓

- Various concentrations of the test drug at 37·c for 20 min in a K/Na

  phosphate buffer (50 mM, pH 7.2), followed by cooling in an ice bath for 45 min.

↓

- To determine non-specific binding, samples containing 2nM (+) Butaclamol are incubated under identical conditions without the test compound.

↓

- Bound ligand is seperated by rapid filteration though Whatsman GF/B glass fiber filters.

↓

- The filters are washed three times with ice-cold buffer, dried, and shaken throughly with 3.5 ml scintillation fluid.

↓

- Radioactivity is determined in a liquid scintillation counter.

↓

- Specific binding is defined as the difference between total binding and binding in the presence of 2.0 mM (+)-Butaclamol.

SCINTILLATION COUNTER

EVALUATION

➢ The following parameters are determined:

- Total binding of H-Spiroperidol
- Non-specific binding: binding of samples containing 2mM Butaclamol
- Specific binding: total binding- non-specific binding
- % inhibition: 100- specific binding as percentage of the control value.