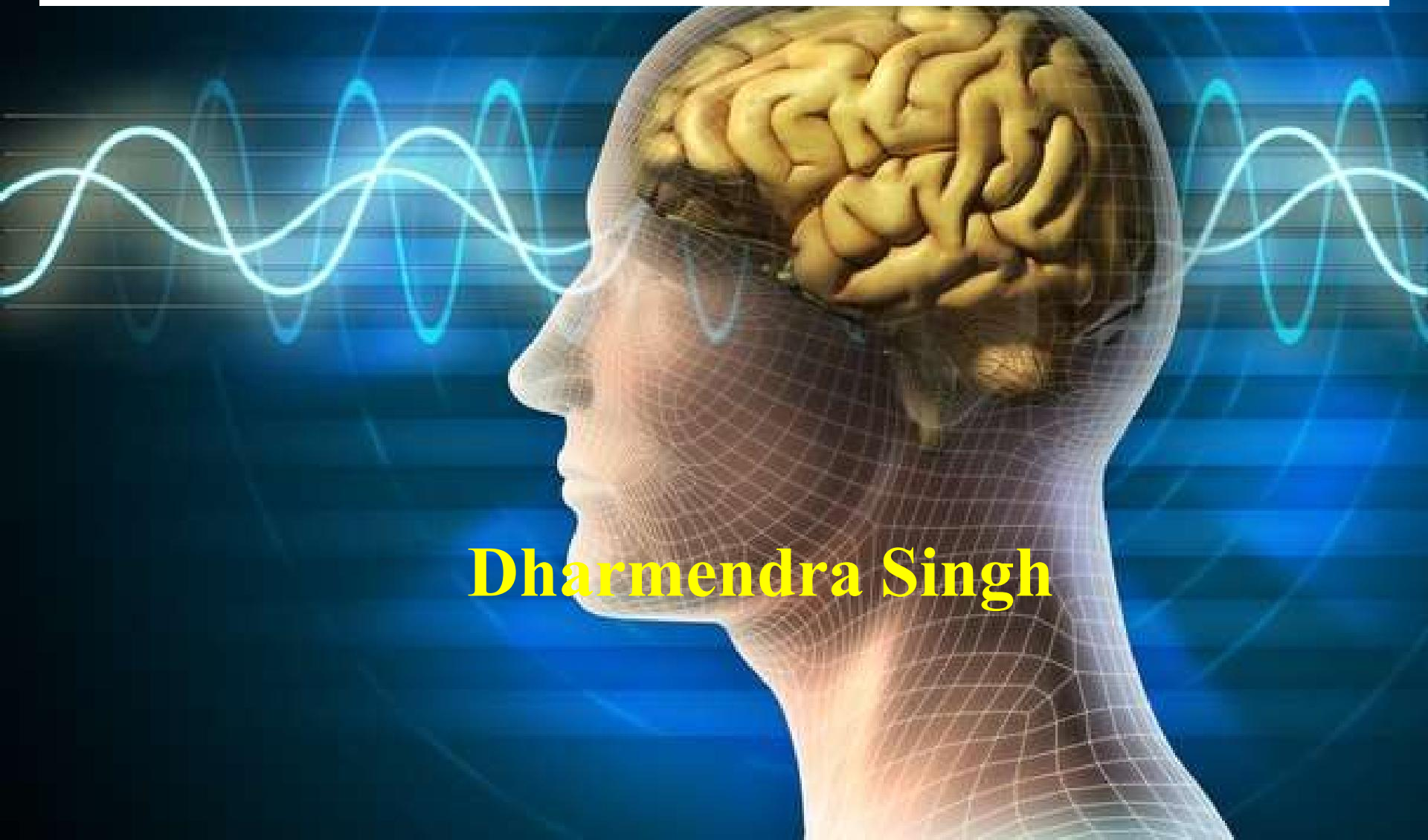
# PHARMACOLOGICAL SCREENING OF ANTI-PSYCHOTIC AGENTS



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# PSYCHOSIS

Psychosis is a thought disorder characterized by disturbances of reality and perception (delusions and hallucinations), impaired cognitive functioning and inappropriate or diminished affect (mood).

Psychosis denotes many mental disorders.

Schizophrenia is a particular kind of psychosis characterized mainly by a clear sensorium but a marked thinking disturbance.

## Psychosis can be broadly Categorized in to four groups

1.**Acute and chronic organic brain syndromes (cognitive disorders)** such as, delirium and dementia, prominent features of confusion, disorientation, defective memory and disorganized behavior.

2.**Functional disorders** such as, memory and orientation mostly retained by emotion, thought, reasoning and behavior are altered.

3.**Schizophrenia (split mind)** i.e.splitting of perception and interpretation from reality- hallucination, inability to think coherently. Schizophrenia is often described in terms of positive or negative symptoms..

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

# SCREENING MODELS

# IN VIVO MODELS

## Behavioral tests

1. Catalepsy in rodents.
2. Golden hamster test.
3. Pole climb avoidance in rats.
4. Conditioned avoidance reflex in rats.

## Tests based on pharmacologic antagonism

1. Inhibition of Amphetamine-induced stereotypy in rats.
2. Inhibition of Apomorphine-induced stereotypy in mice.
3. Phencyclidine-induced bizarre pattern locomotor activity and stereotypy.
4. Neuro developmental model.

# IN VITRO MODELS

1. $D_1$ - receptor assay

2. $D_2$ - receptor assay

3. Binding to $D_3$ - receptor

4. Binding to $D_4$ - receptor

5. $\alpha_1$ - adrenergic receptor binding in brain

6. Measurement of neurotransmitters by intracranial micro dialysis.

7. Binding to sigma receptors

# IN VIVO MODELS

# CATALEPSY IN RODENTS

## 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 0.2 mg/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 fore paw on the bar is determined.

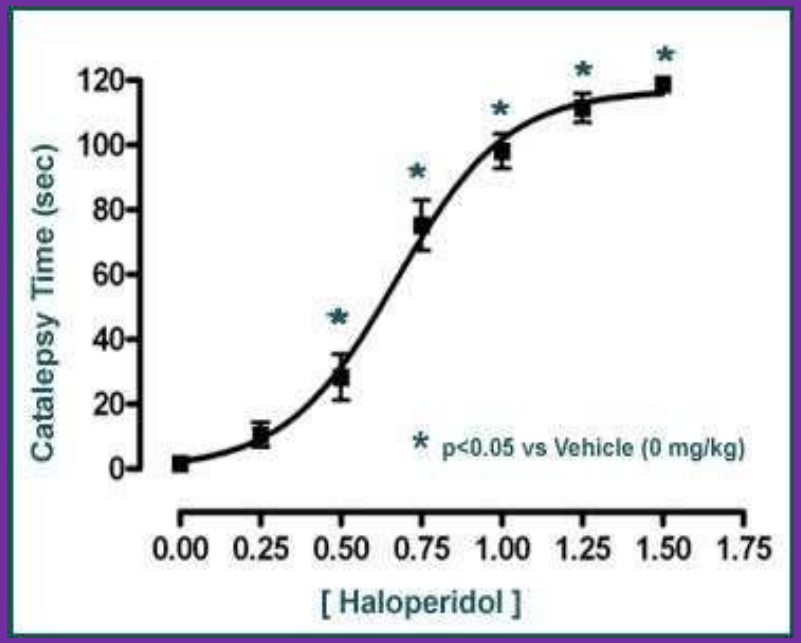
❖ When the animal removes its paws, the time is recorded and the rat is repositioned on the bar.

❖ Four trials 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. $ED_{50}$ values are calculated for comparison & elucidation of potency.

Haloperidol dose dependently increases catalepsy time.



# INHIBITION OF AMPHETAMINE INDUCED STEREOTYPY IN RATS

## Purpose & rationale

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

# PROCEDURE

- **Groupings;**

Group I (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 - 200 g are selected and grouped into 6 each in control and treated.

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

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

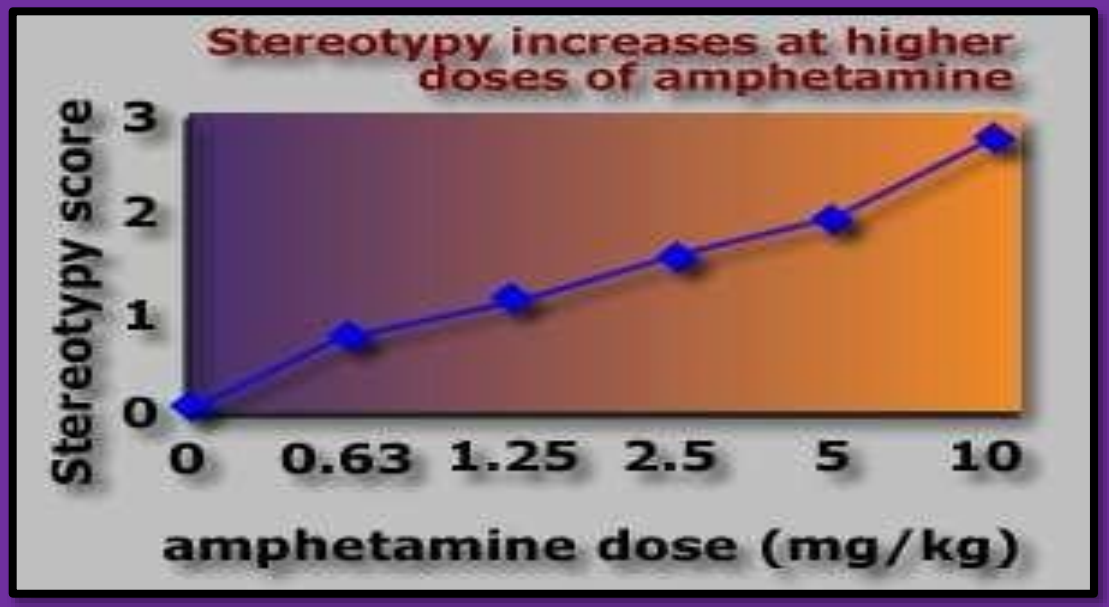
- In the group I animals which have been administered d-Amphetamine shows stereotypic behaviour which is characterized by continuous sniffing, licking or chewing and compulsive gnawing.

- The stereotypic behaviour is reduced or abolished in drug treated group.

## 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 drug is confirmed as neuroleptic agent.



# INHIBITION OF APOMORPHINE INDUCED STEREOTYPY IN RATS

## Purpose & rationale

- Apomorphine (stimulate dopamine receptor in CTZ) induces a stereotyped behavior in rats, characterized by licking, sniffing and gnawing in a repetitive,compulsive manner, which is an indication of striatal dopaminergic stimulation.
- Compounds which prevent apomorphine-induced stereotypy antagonize dopamine receptors in the nigrostriatal system.
- Furthermore, antagonism of this behavior is predictive of propensity (tendency to behave in certain way) for the development of extrapyramidal side effects.

# PROCEDURE

- For screening, group of 6 male Wistar rats with a body weight between 120 and 200 g are used.
- The test drug or the standard are administered I.P. 60 min prior to Apomorphine dosage.
- Apomorphine HCl is injected S.C. at a dose of 1.5 mg/kg.
- The animals are placed in individual plastic cages.
- A 10 sec observation period is used to measure the presence of stereotypic activity such as sniffing, licking and chewing 10 min after apomorphine administration.
- An animal is considered protected if this behavior is reduced or abolished.

## EVALUATION

- The percent effectiveness of a drug is determined by the number of animals protected in each group.
- With a group size of 10 animals dose response curves are obtained and $ED_{50}$ values calculated.
- $ED_{50}$ values were found to be 0.2 mg/kg S.C. for Haloperidol and 5.0 mg/kg for Chlorpromazine, whereas Clozapine was ineffective even at high doses.

# GOLDEN HAMSTER TEST

## 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: [$^3$H]-SPIROPERIDOL BINDING

## 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 ( pH 7.7 ).
- The homogenate is centrifuged at 40 000 rpm for 15 min.
- The pellet is rehomogenized in fresh buffer and recentrifuged at 40 000 rpm.
- The final pellet is then resuspended in Tris buffer.

# Assay

The membrane preparations are incubated with $^{3}$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 2mM (+)-Butaclamol are incubated under identical conditions without the test compound.

↓

Bound ligand is separated by rapid filtration through Whatman GF/B glass fiber filters.

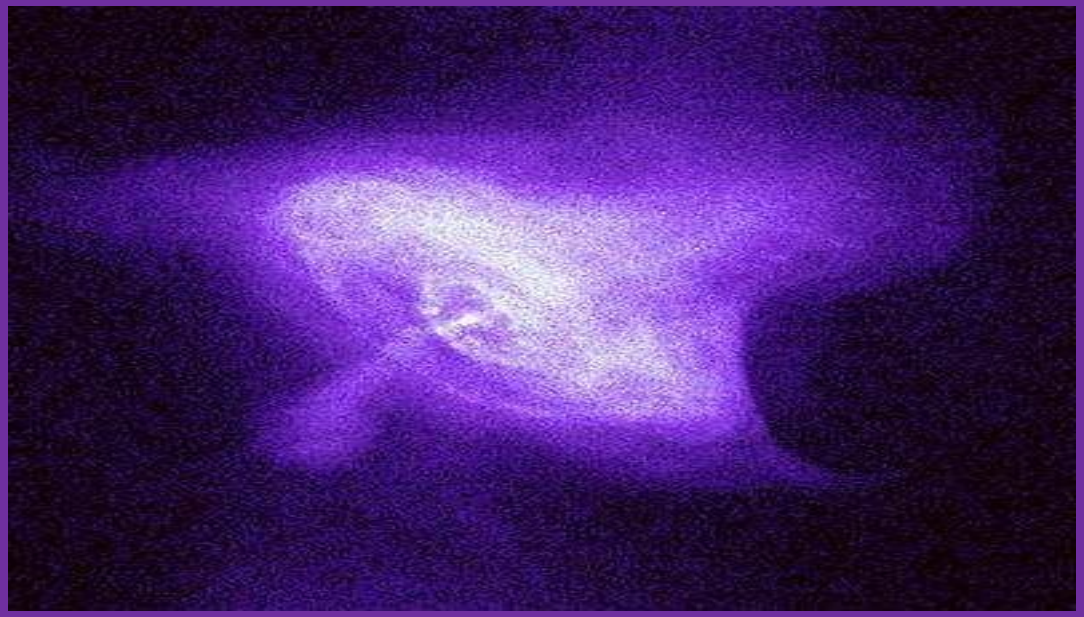
The filters are washed three times with ice-cold buffer, dried, and shaken thoroughly with 3.5 ml scintillation fluid.

Radioactivity is determined in a liquid scintillation counter.

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

# SCINTILLATION COUNTER

# SCINTILLATION FLUID



## EVALUATION

The following parameters are determined:

- Total binding of $^{3}$H-spiroperidol
- Non-specific binding: binding of samples containing 2 mM Butaclamol
- Specific binding: total binding – non-specific binding
- % inhibition: 100 – specific binding as percentage of the control value.

# REFERENCES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2. **Drug screening methods** – SK Gupta.

3. **Essentials of Medical Pharmacology-**KD Tripathi

4. **Internet source**

**The Search for Novel Antipsychotic Drugs-** Paul Kenyon