



## A REVIEW: NEUROPHARMACOLOGICAL SCREENING TECHNIQUES

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### ABSTRACT

This review presents various models to screen various traditional drugs and modern drugs for neurological effects. This brings a compilation make easy to go through the various models at same time and make it easy to screen the drugs. It reveals principle, apparatus and procedure of these models. This screening model can be use to study of memory enhancing, Anxiolytic, Antidepressant activity.

**Keyword:** Scopolamine; Elevated plus maze; Shuttle Box Avoidance.

### INTRODUCTION

The Central nervous system (CNS) comprising of the brain and spinal cord process information with the help of chemical messenger viz. neurotransmitter, neuromodulators, neuroregulators, neuromediators and neurotropic factor which act via specific mechanism to mediate neurotransmission, neurotransmitter viz., nor adrenaline, adrenaline, dopamine, Gamma Amino Butyric Acid (GABA), glutamate, acetylcholine, 5- hydroxytryptamine (5-HT), peptides viz. endorphins, serotonin, glycogen and vasoactive intestinal polypeptides (VIP) etc and neuromodulator viz. prostaglandins (PGs), purines and neuropeptides interact with their recognition sites i.e. Receptor and regulate the function of CNS1.

According to the world health report (WHO 2001) approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receive even the most basic treatment this amount to 12.3% of the global burden of

disease and will rise to 15% by 2020<sup>2</sup>. Drug acting in the central nervous system were among the first to be discovered by the primitive human and are still the most widely used group of pharmacological agents. The CNS acting drugs are invaluable therapeutically, because they can produce specific physiological and psychological effects from the vast array of material medica of the indigenous system so many plants have been reported to have activity against CNS disorders and thus act as very useful remedies for the alleviation of human suffering<sup>3</sup>.

In the search of new therapeutic product for the treatment of neurological disorder medicinal plant research world wide has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models. Many classical animal models of preliminary pharmacological tests of activities on CNS which provide information about action upon psychomotor performance, motor behaviour and neurotoxicity . The

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depression activity gives an indication of the level of the excitability of the CNS and this decrease may be related to sedation resulting from depression of CNS<sup>4</sup>. This review presents various models to screen various traditional drugs and modern drugs for neurological effects. This brings a compilation make easy to go through the various models at same time and make it easy to screen the drugs. It reveals principle, apparatus and procedure of these models.

## **NEUROPHARMACOLOGICAL SCREENING TECHNIQUES**

### **Memory enhancing activity**

Dementia is a mental disorder characterized by loss of intellectual ability sufficiently severe as to interfere with one's occupational or social activities. Dementia is of several types and it invariably involves impairment of memory. The most common cause of dementia is Alzheimer's disease, which is a progressive neurodegenerative disorder associated with loss of neurons in distinct brain areas. The central cholinergic pathways play a prominent role in learning and memory processes<sup>5</sup>. Centrally acting antimuscarinic drugs (e.g. Scopolamine) impair learning and memory both in animals<sup>6</sup> and human beings<sup>7</sup>. Epidemiological studies of Indian population reveal that dementia is largely a hidden problem<sup>8</sup>. Prevalence rates for dementia increase exponentially. With advancing age<sup>9&10</sup>. Since allopathic system of medicine is yet to provide a radical cure, it is worthwhile to look for new directions, which would minimize the memory loss seen in elderly patients. Various laboratory models for testing learning and memory are

1. Scopolamine induced amnesia (Interoceptive Behaviour Model).
2. Diazepam induced amnesia (Interoceptive Behaviour Model).
3. Elevated plus maze (Exteroceptive Behaviour Model).
4. Shuttle box avoidance (Two - way shuttle box)
5. Passive avoidance paradigm (Exteroceptive behaviour models)

### **Scopolamine induced amnesia in mice Principle**

The administration of the antimuscarinic agent scopolamine to young human volunteers produces transient memory deficits<sup>11</sup>. Analogously, scopolamine has been shown to impair memory retention when given to mice shortly before training in a dark avoidance task<sup>12, 13 & 14</sup>. 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<sup>15</sup>.

### **Procedure**

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 scopolamine test is performed in groups of 10 male NMRI mice weighing 26-32 g in a one-trial, passive avoidance paradigm. Five minute after i.p. Administration of 3 mg/kg scopolamine hydrobromide, each mouse is individually placed in the bright part of a two - chambered (bright and dark) apparatus for training.

After a brief orientation period, the mouse enters the second darker chamber. Once inside the second chamber door is closed which prevents the mouse from escaping, and a 1 mA, 1 - sec 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. Latency in entering the second darker 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 latency about of 250 sec, treatment with scopolamine reduces the latency to 50 sec.

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. 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 doses - response curves can be established whereas with other drugs inverse U- shaped dose-responses are observed.

**Elevated plus - maze  
(exteroceptive behavior model)**

**Principle**

Out of many possibilities to modify maze tests e.g. water maze<sup>16</sup>, the Y-maze, the radial maze<sup>17</sup>, and the elevated plus maze<sup>18&19</sup> have found acceptance in many laboratories. The test has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration time; anxiogenic compounds have the opposite effect.

**Procedure**

Elevated plus maze served to evaluate learning and memory in mice. The procedure, technique and end point for testing learning and memory is followed as per parameters described by the investigators working in the area of neuro psychopharmacology. The apparatus consisted of two open arms (16cm x 5cm) and two closed arms (16cm x 5cm x 12cm). The arms extended from a central platform (5cm x 5cm) and the maze is elevated to a height of 25 cm of the floor. On the first day, such mouse is placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) is taken by mouse with all its four legs to move into one of enclosed arms. TL is recorded on the first day. If the animal did not enter arms within 90sec. It is gently pushed into one of two enclosed arms and TL is recorded at 90s. Then three consecutive mice are allowed to explore the maze for another 10s and then returned to its home cage. Retention of this learned - task is

examined 24H after the first day trial. Another laboratory model viz. Passive avoidance apparatus was employed to substantiate the findings and overcome the limitations of the elevated plus - maze.

**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 shuttle box can be easily automated<sup>20</sup>.

**Procedure**

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Rats of both sexes are used and maintained under standard conditions. The apparatus used consist of a rectangular box 50 x 15 cm<sup>2</sup> 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 (10x10cm<sup>2</sup>) into two 25x15cm<sup>2</sup> compartments. Each compartments can be illuminated by a 20 W bulb mounted in the hinged Plexiglas lids. A fixed resistance shock source with an automatic switch (0.5 sec on 1.5 sec off) is used. Simple programming equipment provides for automatic delivery of the command stimulus (CS) and the unconditioned stimulus (US). The apparatus is placed in a dimly lit room with a masking noise background (white noise) of 60 dB. The animal is allowed to explore the apparatus for 5 minutes with the connecting door open and the compartment lights switched off. The guillotine door is then closed. 20 sec the light is switched on in the compartments containing the animal and the door is opened. A tone (CS) is presented and 5 sec later the floor shock is applied in this illuminated compartment and continued until the animal escapes to the dark side of the compartment; the connecting door is close and the shock discontinued.

After in variable inter trial (ITI; 30-90 Sec) the light is switched on in previous dark compartment, the door is opened and animal is required to cross to another side. The training is continued until animal reaches critical of 9 avoidances in 10 consecutive trials. Retention is tested at different interval after the original training by the retaining the animal to same criterion again.

The animal need to reach the safe on both days is measured. In addition the number error (not reaching the safe area) is recorded. The task is rather difficult due to lack of permanent safe area 'lack of simple instrumental response, presences of variable aversive gradient and increased weight of emotion factor.

### **Passive avoidance paradigm (exteroceptive behavior model)**

#### **Principle**

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 behaviour.

#### **Procedure**

Passive avoidance behaviour based on the negative reinforcement used to examine the long term memory<sup>21, 22</sup>. The apparatus considered of a box (27 cm x 27 cm x 27 cm) having three vales of wood and one wall of Plexiglas, featuring a grid floor (3 mm stainless steel rods set 8mm apart), with a wood platform (10cm x 7cm x 1.7cm), in center of grid floor. The box is illuminated with 15W bulbs during experimental period. Electric shock (15V AC) is delivered to the grid floor. Training is carried out in two similar sessions. Each mouse gently placed on wood platform set in center grid floor. When the mouse stepped down place all its paws on the grid floor, shock is delivered to 15 sec and step - down latency (SDL) is recorded. SDL is defined as time taken by mouse to steep down from wood platform to grid floor all its paws on grid floor.

Animal showing the SDL in rang (2-15 sec) during first test are used for the second session and the retention test. The second session carried out at 90 min after the first test. When animal stepped down for period of 60 sec retention is tested after 24h in the similar manner, except electric shock is not applied to grid floor. Each mouse again place on the platform and the SDL is recorded, with the upper cut of time of 300 sec.

#### **Anxiolytic Activity**

##### **Principle**

The animal model is considered one of most widely validated tests of assaying sedative and anxiolytic substances such as benzodiazepines. The test drug induced anxiolytic effect beginning at lower doses employed. An increase of most important variables of EPM test was found as follow; the percentage of time of mice spend on the open arms as well as the percentage of entries in the dark arms. The anxiolytic effect is also evidenced through light and dark test. As with the EPM test, this model is useful for modeling of anxiety. The low dose dependent effect could be attributed to biological variability, as well as chemical complexity of the test drug. Various model of anti anxiolytic testing are<sup>23, 24</sup>.

1. Elevated plus-maze model (EPM).
2. Forced swimming test (FST).
3. Light – dark test (LDT)
4. Open – field test (OFT)

##### **Elevated plus – maze (EPM)**

This has widely validated to measure anxiety to rodents. This apparatus is made of Plexiglas and consisted to two open arms (30cm x 5cm) with 30cm walls. The arms extended from the central platform (5cm x 5cm). This wall is elevated 38.5cm from the room floor. The each animal is placed at the center of maze, facing one of the enclosed arms. Number of entries and time spend in enclosed and open is recorded for 5 min test. Entry an arm is defined as animal placing all four paws onto the arm. All tests are tapped by using a video camera. After each test, the maze is carefully cleaned up with wet tissue paper (10% ethanol solution).

### Forcing swimming Test (FST)

The FST is most widely used pharmacological *in vivo* model assessing anti depressant activity. The development of immobility when mice are placed in inescapable cylinder fill with water reflects cessation of persistent escape directed behavior. The apparatus consist of clear Plexiglas cylinder (20cm height x 12cm diameter) filled to a 15cm depth within the water (25°C) in the pre- test session; every animal is placed individually into cylinder for 15min. 24h prior to the 5min swimming test the test drug and distilled water are administered three time, immediately after 15min pre - test, 18 and 1h prior to the swimming test. During the test session a trained observer registered the immobility time, considered to be when the mouse made no further attempts to escape, apart from the movements necessary to keep its head above the water. It is suggested that the immobility reflected a state of lowered mood in which the animals had given up hope of finding an exit and had resigned themselves to the experiments situation.

### Light dark test (LDT)

The apparatus consists of a Plexiglas box with two compartments (20 x 20cm) one of which is illuminated with a white light while the other remained dark. Each animal is placed at the centered of the illuminated compartments; facing one of the dark places, as well as the number of entries in each space is recorded for 5min.

### Open field test (OFT)

Open field test area is made of acrylic transparent walls and black floor (30 cm x 30 cm x 15 cm) divided into nine square of equal area. The open field is used to evaluate the exploratory activity of animal<sup>25</sup>. The observed parameters are the number of square crossed (with the four paws) and number of rearing.

### Antidepressant activity

Antidepressant activity was indicated the mood elevating due to various mechanism of the antidepressant drugs, such as inhibition of the enzyme of monoamine

oxidase, inhibition of reuptake bioamines and enhancement of the concentration of 5-HT. Later on, inhibition of reuptake of bioamines was found to be main mechanism of action to down regulation of  $\beta$  receptor 26. Several lines of preclinical and clinical evidence indicates that enhancement of 5-HT mediated neurotransmission might underline the therapeutic effect of most of the anti depressant. This behavioral effect very similar to that found by other author after treating mice with classical anti depressant drugs as IMI<sup>27</sup>. Various models for antidepressant activities are as follows.

1. Despair Swim Test
2. Learned helplessness test
3. Muricide behaviour in rats

### Despair swim Test

#### Principle

Behavioural despair was proposed as a model to test for antidepressant activity. It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behaviour of immobility. This behaviour reflects a state of despair which can reduce by several agents which are therapeutically effective inhuman depression<sup>28, 29</sup>.

#### Procedure

Male Sprague - Dawley rats weighing 160–180g are used. They are brought to the laboratory at least one day before the experiment and are housed separately in Makrolon cages with free access to food and water. Naive rats are individually forced to swim inside a vertical. Plexiglas cylinder (height: 40cm; diameter: 18cm, containing 15cm of water maintained at 25°C). Rats placed in the cylinders for the first time are initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2–3min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. After 5–6min immobility reaches a plateau where the rats remain immobile for approximately 80% of the time. After 15 min in the water the rats are removed and allowed to dry in a heated enclosure (32°C) before being returned to their home cages.



They are again placed in the cylinder 24 h later and the total duration of immobility is measured during a 5 min test. Floating behaviour during this 5 min period has been found to be reproducible in different groups of rats. An animal is judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. Test drugs or standard are administered one hour prior to testing. Since experiments with the standard drug (imipramine) showed that injections 1, 5 and 24 h prior the test gave the most stable results in reducing floating these times are chosen for the experiment.

### Learned helplessness in rats

#### Principle

Animals exposed to inescapable and unavoidable electric in one situation later fail to escape shock in a different situation when escape is possible<sup>30</sup>. This phenomenon was evaluated as a potential animal model of depression<sup>31, 32</sup>. On day 19<sup>th</sup> of the investigation rats are subjected to foot shock (60 scrambled shocks, 15 s duration, 0.8 mA, every min) in a two compartment jumping box with the escape door to the unelectrified adjoining compartment closed. The exercise continued for one h. On day 21, 48 hour afterwards the rats are subjected to avoidance training using the same apparatus but keeping the escape route to the unelectrified chamber open. During this avoidance training, the rats are placed in the electrified chamber allowed to acclimatize for 5 min before being subjected to 30 avoidance trials with an intertribal interval of 30s. During the first 3s of trials a buzzer stimulus is presented followed by electro shock through the grid floor for the next 3s. The avoidance response, characterized by escape to the adjoining "safe" chamber during conditioned stimulus, is noted. Failure to escape during unconditioned stimulus within 15s is assessed as "escape failure".

### Muricide behavior in Rats

#### Principle

Horovitz et al. described a selective inhibition of mouse-killing behaviour in rats

by antidepressants<sup>33</sup>. The test can be used to evaluate antidepressants such as tri-cyclic and MAO inhibitors.

#### Procedure

Male Sprague-Dawley rats (300 – 350 g) are isolated for 6 weeks in individual cages. They have access to food and water ad libitum. One mouse is placed into the rat's cage. About 10 to 30% of rats kill the mouse by biting the animal through the cervical cord. Only rats consistently killing mice within 5 min after presentation are used for the test. The mice are removed 15 to 45s after they have been killed in order to prevent the rats from eating them. Drugs are injected i.p. to the rats before the test. Mice are presented 30, 60 and 120 min after drug administration.

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