Experimental methods for evaluation of psychotropic agents in rodents: II-Antidepressants

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Rodent models of clinical depression are extensively used for the evaluation of putative antidepressants. In the present review, the available experimental methods which can be utilized by most laboratories involved in preclinical screening of antidepressants, have been discussed. The methods have been categorized on the basis of induction of the depressive state or on the assumption that monoamine deficiency leads to depression. These methods have been critically validated in terms of efficacy of standard antidepressants in these tests and, in some cases, by the neurochemical basis of depression, namely, the deficient monoaminergic theory of clinical depression.

Depression is a chronic illness that affects people of all ages. The lifetime risk of major depression disorders in community samples varies from 10-25% for females and 5-12% for males. Although there are many effective antidepressants available today, the current armamentarium of therapy is often inadequate, with unsatisfactory results in about one third of all subjects treated. This provides impetus in the search for newer and more effective antidepressants. As our understanding of the biochemical basis of endogenous depression proceeds beyond the confines of the classical monoaminergic deficiency hypothesis, to involve other neurotransmitter systems, including serotonergic, cholinergic and peptidergic neuronal activity, the search for newer antidepressants continues to expand and diversify. The large number of new antidepressants introduced in recent years into clinical practice have, unfortunately, failed to surpass the plateau of efficacy exhibited by the classical antidepressant agents, the monoamine oxidase (MAO) inhibitors and tricyclic antidepressants (TCAs). Ideally, a new antidepressant should not only have greater efficacy and be cost-effective, it has to have a rapid onset of action. The expected delay in clinical efficacy of most of the antidepressants available today is between 10 days to 3 weeks. The search for such an ideal antidepressant as a more effective medication, with better tolerability, rapid onset of action and ease of dosing, requires valid preclinical tests which can predict clinical antidepressant activity. The plant kingdom may be a part of this search for the 'Holy Grail' of antidepressant psychopharmacology, as evidenced by the emergence of Hypericum perforatum (St. John's wort) as an effective clinical antidepressant.

Like any other field in psycho-pharmacology, preclinical research on anti-depressants is confounded by the virtual impossibility of developing investigative animal models of clinical depression. The major problem faced by all animal models of depression is that core features of depression - the depressed mood, feeling sad, neglected and melancholic, as well as the suicidal tendency, cannot be modelled in animals. Some criteria have been suggested for the validation of animal models of psychiatric disorders, namely predictive, face and construct validity. These have been discussed at length in a recent review on evaluation of anxiolytic agents. In brief, the animal model used should be able to predict clinical use, have some behavioural and neurochemical similarity with clinical depression, and clinically effective antidepressants should be effective in the animal model as well. Thus, these models cannot only predict antidepressant activity, but also provide an insight into the psychobiology of depression. A variety of animal models can be used to detect antidepressant activity. It is always imperative that a battery of tests be used to evaluate and confirm preclinical antidepressant activity.

Methods

Animals—Adult rats and mice are used. Several investigators have used only male rodents, although there does not appear to be any significant degree of sex variation in the induction of depression models. However, it is always advisable to ensure that the animals belong to a specific species and are inbred, to avoid variability of the results.

Drugs, route of administration and duration of treatment—It is always advisable to administer the test drug orally, in graded doses, since this will be likely route of clinical use. However, in case of a new drug, with unknown pharmacokinetic profile, the intraperitoneal route can be used for initial experimentation. A vehicle treated control group is important for the sake of statistical evaluation. Single acute drug administration can be adopted. However, in case of plant extracts, subchronic (3-5 days) drug administration may be required.

Standard antidepressants—The standard drug used is usually imipramine (10 mg/kg, ip pretreatment time 30-45 min). However, a MAO inhibitor like nialamide (10 mg/kg, ip pretreatment time 2 hr) may be used if this is the likely mode of action of the test drug. It is important to note that, while classical tricyclic antidepressants like imipramine can...
be effective both on pre- and post-treatment, the MAO inhibitors are effective only on pretreatment.

Animal models of depression—Basically two types of rodent behaviour models are used. The first type uses drugs to induce depression or depends upon drug-induced behaviours, and the other type utilizes behavioural models which have some resemblance to endogenous depression.

I. Pharmacological models

(A) Reserpine (or tetrabenazine) syndrome—Reserpine depletes central and peripheral monoamines, whereas tetrabenazine has a selective central action. These drugs induce a syndrome (ptosis, hypothermia, catalepsy and decreased locomotor activity), the reversal of which is used as a reliable initial method to detect antidepressant activity. Reserpine (2.5-5.0 mg/kg, sc) or tetrabenazine (10 mg/kg, ip) are used and the pharmacological effects are assessed 2 hr later. TCAs are effective in attenuating the syndrome both on pre- and post-treatment, whereas MAO inhibitors are effective only on pretreatment. In addition, the proconvulsant, blockade of conditioned avoidance response in rodents, and emetic (pecking) response in pigeons, have also been used as test parameters.

a. Reserpine-induced ptosis—Ptosis of palpebral closure is graded from 0 to 4, 0 being complete closure and 4 indicating that the eyes are widely open.

b. Reserpine-induced decrease in locomotor activity—Reduction in locomotor activity is best tested in an automated activity cage recording photobeam breaks as counts. Rats are placed individually and 10 min counts are recorded for 30 min, to obviate the initial locomotor spurt in ambulatory behaviour.

c. Reserpine-induced hypothermia—Rectal temperature, considered as the core body temperature, can be recorded using a multichannel telemeter. The animal is placed in a loosely fitting perspex chamber and a thermometer probe is inserted 4 cm deep into the rectum (in rats), and kept in situ for the duration of the experiment. Temperature responses are noted before and 1, 2 and 4 hr after reserpine administration.

d. Reserpine-induced catalepsy—A variety of methods can be used to assess catalepsy. The Petwee's ring test has been shown to be sensitive in distinguishing between MAO A and MAO B inhibitors. The rat is placed on an iron ring (diameter 12 cm) fixed to a steel stand at a height of 15 cm. The time during which the rat remains motionless, with the complete cessation of snout and whisker movements, out of a total observation period of 5 min, is used to calculate percent immobility. A smaller ring (6 cm) can be used for mice.

The method is simple, rapid and reliable, and can detect all classes of antidepressants. Rats provide more cogent data than mice. However, false negatives (mianserin) and false positives (methylidopa, antihistaminics) are on record.

(B) Amphetamine potentiation—Most antidepressants, including TCAs and MAO inhibitors potentiate the central actions of amphetamine, including hyperthermid, augmented locomotor activity, stereotypy and lethality in aggregated rodents.

a. Amphetamine-induced stereotypy—Amphetamine (5-10 mg/kg, ip) induces a stereotyped behaviour which is best noted 30 and 60 min after administration in rats. The rat is placed in a spacious cage in a dimly-lit quiet room. The latency of onset, intensity and duration of stereotypy, are assessed and scored:

1. Discontinuous sniffing, constant exploratory activity
2. Continuous sniffing, periodic exploratory activity, small head movements
3. Continuous sniffing, small body and head movements, discontinuous gnawing, biting and licking the cage wall, brief spurts of locomotor activity
4. Continuous gnawing, biting and licking of cage wall, no ambulation except for occasional backward movements.

b. Amphetamine-induced hyperthermia—Temperature changes are recorded at 30 min intervals for 2-4 hr after administration of amphetamine (2.5-5.0 mg/kg, ip) by a telemeter, as mentioned earlier, recording rectal temperature.

c. Amphetamine-induced increase in locomotor activity—Locomotor activity is recorded in an automated activity cage, as mentioned earlier, at 10 min intervals for 30 min, 2 hr after amphetamine (1-2 mg/kg, ip).

d. Amphetamine-induced toxicity in grouped rodents—Mice are usually used and groups of 10 mice are kept crowded in a small wire mesh cage (16 cm³). 30 min later amphetamine (10 mg/kg, ip) is administered to the animals and lethality is recorded 4 hr later. Results are expressed as percentage increase in amphetamine lethality as compared to vehicle-treated controls. This dose of amphetamine induces 20-30% mortality in mice. The relatively newer antidepressants, mianserin and trazadone are ineffective in this test.

(C) Apomorphine antagonism—Apomorphine in higher doses (16 mg/kg, ip) induces hyperthermia which is not antagonized by dopamine receptor blocking neuroleptics which can, however, attenuate apomorphine-induced stereotypy and climbing behaviour. A wide range of antidepressants can, on the contrary, reverse the hyperthermia. This method is simple and useful for rapid detection of antidepressant activity.

(D) Potentiation of tryptamine-induced convulsions—Tryptamine (60 mg/kg, ip) produces bilateral clonic convulsive movements of fore paws in mice, characterized by pronounced up and down movements of the paws which push the animals backwards, resulting in retro-pulsion. TCAs and MAO inhibitors potentiate a dose of tryptamine (15 mg/kg, ip) which produces minimal convulsions.

(E) Yohimbine potentiation—Yohimbine (25 mg/kg, sc) an antagonist at adrenergic alpha-2 receptors, produces minimal lethality in rodents. However, TCAs, MAO inhibitors and most of the newer antidepressants, potentiate the lethality of this dose of yohimbine in rats and mice.
This test has been used as a simple and rapid test for screening diverse groups of antidepressants.

(F) Potentiation of 5-hydroxytryptophan (5-HTP) responses—5-HTP, the 5-HT precursor, produces typical behavioural responses in rodents. The effect in rats, designated 'wet dog shakes', comprising of intermittent body responses—5-HTP, twitches at three two minute intervals (19-21, 23-25 and movements including a shaking of the head, whereas, in mice, the effect is predominantly rapid and intermittent head-twitches [12,13]. In mice, a dose of SHTP (50 mg/kg, ip) which produces minimal head-twitch response is used, and the potentiation induced by drugs is noted by counting the twitches at three two minute intervals (19-21, 23-25 and 27-29 min) after 5-HTP administration. The final data is presented as the mean of the head-twitches during the test periods [4]. This model can predict anti-depressants influencing 5-HT activity, namely selective 5-HT reuptake inhibitors, like fluoxetine, and 5-HT selective TCAs, like chlorimipramine.

(G) Post-swim grooming response—Groups of mice are placed in a water bath containing water (32°C, depth 10 cm) for 3 min. Thereafter, the animals are removed and observed for grooming behaviour, every min, during a 10 sec period, for 30 min. A score of one is given if the mouse was grooming during the 10 sec observation period or scored as 0 if not grooming. Thus each mouse could exhibit a maximum score of 30. This test, being a dopamine-mediated response, can be used to detect MAO B inhibitor activity [14].

(II) Potentiation of 1-DOPA induced response—Mice are treated with 1-DOPA (25 mg/kg, ip) and benserazide hydrochloride (6.5 mg/kg, ip). The change in behaviour is scored every 10 min for 30 min. A scoring system, ranging from 1 to 4, is used, based on the following criteria: 1=piloerection, minimal increase in ambulation, 2=piloerection, marked ambulation, salivation, irritability, 3=piloerection, profuse salivation, jumping, vocalization, increased ambulation, 4=piloerection, profuse salivation, stereotypy (compulsive gnawing and biting the cage wall), reduced ambulation, aggressive behaviour and automutilation (biting of tail and fore paws). The dose of 1-DOPA used produces minimal behavioural changes, which are potentiated by all classes of antidepressants except those acting selectively through increased 5-HT activity [5].

(I) Muridical behaviour in rats—Muridical, or compulsive mouse killing behaviour, was noted in female rats of the Holtzman strain which instinctively attacked and killed mice irrespective of their safety status [15]. However, a small percentage of other rat species can also exhibit muridical behaviour (20% in Charles Foster and Wistar strains). Rats are prescreened for muridical behaviour, 48 hr before the test and the behaviour is confirmed 24 hr later. The percentage of rats exhibiting muridical behaviour within 30 sec of introduction of a mouse into the rat cage is noted. Antidepressants attenuate muridical behaviour at doses below that inducing motor incoordination (as tested by the rota-rod method). Other psychotropic agents, including neuroleptics, block muridical behaviour only at dose levels inducing motor deficit. A major precaution is to remove the mouse carcass promptly to prevent it being eaten up by the killer rat. Rats exhibiting muridical behaviour can be reused but they have to be caged individually in isolation [5]. Non-muridical rats can be rendered muridical by pretreatment with picrocarpine (2.5 - 5.0 mg/kg, ip) [7].

II. Behavioural models

These models are based on manipulations of social reinforcement or environmental unpredictability. The induced behavioural responses of the animal is postulated to represent clinical depression [6].

(A) 'Behavioural despair' test [16,17]—This model is based on the premise that, when rats or mice are forced to swim in an apparatus from which there is no escape, they will, after initial frenzied attempts to escape, adopt a characteristic immobile posture and make no further attempts to escape. The movements made by the rodent are those necessary to keep its head above the water level. It is postulated [16,17] that the immobility exhibited by the animal reflects a state of 'despair', resigning itself to the experimental situation. In a standard protocol, the rat is placed in a plexiglass chamber (45 x 40 x 30 cm) with water level of 25 cm (25±2°C), so that it cannot touch the bottom with its hind paws or tail, and climb over the edge of the apparatus. The animal is allowed to swim for 10 min, during which it makes several attempts to escape and swims vigorously. Thereafter, the total period of complete cessation of swimming with the head floating just above the water level, is noted during the next 5 min period. The protocol is same in mice except that the vessel dimensions (35 x 15 x 10 cm) and water level (15 cm) are different. The classical TCAs reduce immobility time and a significant correlation exists between the experimental and clinical potencies of these drugs. However, antidepressants acting selectively on the 5-HT system are generally inactive in this test and false positive are induced by opiates and anti-histaminics.

(B) 'Learned helplessness' test—This model is based on the assumption that, exposure to uncontrollable stress associated with repeated experiences of failure to escape from the stress, produces a 'helpless' situation, which results in performance deficits in subsequent learning tasks [18,19]. A typical experiment involves two parts:

a. Inescapable shock treatment—Rats are subjected to footshocks in a two compartment jumping box with the escape route to the adjoining electrified 'safe' chamber closed. A constant current shocker is used to deliver 60 scrambled shocks (15 sec duration, 0.8 ma every min) through the steel mesh grid floor. Control animals are placed in the chamber for 1 hr without experiencing shocks. This exercise is repeated 48 hr later on day 3.

b. Conditioned avoidance training—On day 3, after the second inescapable shock treatment, the rats are subjected to avoidance training where a rat is placed in the electrified chamber and allowed to acclimatize for 5 min before being subjected to 30 avoidance trials, with an inter-trial interval of 30 sec. During the first 3 sec of each trial, a buzzer...
stimulus or a light signal (conditioned stimulus, CS) is presented, followed by footshock (0.8 mA for 3 sec duration, unconditioned stimulus, UCS). The avoidance response is characterized by escape to the adjoining unelectrified chamber during CS, and is designated 'escape response'. Failure to exhibit escape response during CS is assessed as 'escape failure', which is said to represent depressive behaviour. Antidepressants reduce or even eliminate escape failures. This model has excellent predictive validity and is extensively used to screen antidepressants, investigate their mode of action and to evaluate the neurobiology of depressive illness. Rats subjected to inescapable shock also exhibit decreased ambulation and aggression, and loss of appetite with weight loss, which can be utilized as additional investigative parameters.

(C) Isolation-induced hyperactivity—Rats are housed singly in a cage (38 x 26 x 20 cm) in comparison to the normal housing of 4-5 rats/cage. It is ensured that the socially-deprived rats are kept in isolation without visual or auditory contact with their normally housed counterparts, for a period of 10-14 days. Thereafter, the isolated rats are subjected to behavioural testing. Apart from locomotor activity (reduced spontaneous locomotor and exploratory behaviours) and open-field behaviour (reduced activity, rears and immobility), a stereotyping rating, taken for 10 min, on a 0-6 point scale, has been used. The ratings indicate 0—inactivity or sleep, 1—active but decreased response to external stimuli, 2—increased ambulation with bursts of stereotyped sniffing or rearings, 3—marked stereotyped behaviour maintained over a wide area of the cage, 4—marked stereotyped behaviour, mainly sniffing and rearing confined to one location of the cage, 5—marked stereotyped behaviour of licking and gnawing of cage wall, confined to one location of the cage, and 6—assumption of bizarre ‘awkward disjunctive’ postures. Classical and the newer anti-depressants, like mianserin, trazadone and fluoxetine, abolish isolation-induced behaviours.

(D) Tail suspension test—This test is a variant of the behavioural despair test in which immobility is induced by simply suspending a rat or mouse by the tail. Unlike behavioural despair, there is no hypothermia and the behavioural changes last longer than the test period. Mice provide better results and in a typical experiment a mouse is hung on a wire in an upside down posture so that its nostril just touches the water surface in a container. After initial vigorous movements, the mouse assumes an immobile posture and the period of immobility during a 5 min observation period is noted. This test is a reliable and rapid screening method for antidepressants, including those involving the serotonergic system. However, MAO inhibitors are usually inactive.

(E) Chronic unpredictable stress—One of the major consequences of chronic stress is behavioural depression in rodents. Several models have been used, mainly in rats, where, apart from chronicity of the stress, its unpredictability and inability to cope with the stressor, are major factors. In one of the techniques, rats are exposed randomly to a variety of stressors during a 3 week period. The stressors include mild electric shock, immersion in cold water and reversal of light/dark cycle. Another method utilizes a chronic mild stress situation extending over 3-4 week period involving exposure of the rat to overcrowding, wet saw dust on the cage floor, tilting of the cage, flashes of light or loud sound, and intermittent removal of food and water. However, a much simpler form of chronic unpredictable stress, which consistently induces depression and other behavioural changes, including attenuation of male sexual activity and cognitive dysfunction, is daily exposure of rats to 1 hr of foot or tail shock (2 mA, duration 3-5 sec) for a period of 2-3 weeks. The intervals between the shocks is randomly programmed (unpredictability) between 10 and 100 sec. Control animals are placed in the test chamber but receive no shocks. All these methods use the increase in plasma corticosterone as the stress indicator. The methods are sensitive to antidepressants of all classes and MAO inhibitors.

An alternative chronic stress model of depression, shown to have predictive validity and reliability, but not construct validity, involves exposure of rats or mice, sequentially over a period of weeks, to a variety of mild stressors, and the measure most commonly used to evaluate consequent depression is the decrease in consumption of a palatable sweet solution. The generalised decreased in responsiveness to rewards is comparable to anhedonia, the core symptom of the melancholic subtype of major depressive disorder. In a typical experiment, the rodent is exposed sequentially to a variety of mild stressors, including overnight illumination, periods of food and water deprivation, cage tilt, change of cage mate, periodic loud noise and change in the size of the home cage, which change every few hours over a period of weeks or months. The effectiveness of this procedure is usually monitored by tracking, over repeated tests, a decrease in the consumption of and/or preference for a palatable weak (1-2%) sucrose solution. However, other end points, including parameters used in the chronic unpredictable mild footshock technique, can also be investigated. Typical and atypical antidepressants can restore normal behaviour and perturbed physiological functions. The basic defect of this test, despite its utility in evaluating antidepressant activity, and the reason for its limited use, is the chronicity of the model and the difficulty to set it up in a new laboratory. Considerable inter-laboratory variations also preclude wide acceptance of the model. Furthermore, the predictive, validity of this method does not appear to be superior to the widely used Porstot's or the learned helplessness tests. Some investigations have questioned the reliability of this model since clinically effective antidepressants may fail to reverse the chronic stress induced physiological perturbations.

(F) Separation models—The rodent model was evolved on a primate model involving separation of the mother from its progeny. The initial stage of 'protest' (agitation, insomnia, diastress calls and screaming) is followed after 1 to 2 days by 'despair' (decreased normal ac-
tivity, loss of appetite, reduced social interaction and vocalization). TCAs selectively reduce the signs of despair. The same protocol is followed in rats and the model is regarded as one of the best methods with face and construct validity.

(G) Incentive disengagement—Rats are trained in a runway for food reward and then switched to non-reward situation (extinction of learning). Non-rewarded trials are followed by augmented locomotor activity in the first week, and by reduced locomotor activity in the second week. Although the method has good predictive validity, it has not proved popular because of procedural problems.

(H) Disturbed circadian rhythm—Rodents are nocturnal animals, with high locomotor activity at night and relatively low activity during the day. Readjustment to a normal circadian cycle of locomotor activity following reversal of the light-dark cycle is expedited by subchronic (3-5 days) administration of TCAs.

(I) Operant-reward test—This model is based on the premise that hungry rats, trained to press a lever for food reward, find it difficult to desist from lever pressing if the reward is dependent upon the waiting. In a typical test, animals are trained to wait for 72 sec between lever presses to receive food reward. TCAs characteristically increase the number of food rewards that the rat earns under this schedule since lever pressing is closer to the original wait (72 sec) programme. The newer antidepressants and MAO inhibitors are also active in this test.

(J) Other methods—Some other techniques have been used to evaluate antidepressant activity which may not be possible to replicate in most laboratories, given the sophistication of the methods. They are:

a. Olfactory bulbectomy—Bilateral destruction of the olfactory bulbs in rats, leads to impairment of learning, irritability, hyperactivity and increased plasma corticosterone levels. The lag period for the appearance of the behavioural changes is 2-5 weeks and the technique has excellent face validity, being attenuated by all classes of antidepressants.

b. Intracranial self-stimulation—Experimental induction of depression invariably leads to performance deficits in the performance of rewarded behaviours. Attempts were made to identify the brain centre responsible for reward, using implanted intracranial self-stimulation electrodes. The ability of different classes of drugs to increase the frequency and duration of self-stimulation, provides an index of antidepressant activity. Although, all classes of antidepressants are effective, the model lacks specificity.

Conclusion

The importance of pharmacological techniques in preclinical screening of antidepressants cannot be minimized. However, wherever possible, they have to be complemented by corroborative biochemical paradigms. Thus, MAO inhibitor activity, including delineation of selective MAO A and MAO B inhibiting activity, using relevant substrates and enzyme sources, can be performed in most laboratories. Similarly, the specificity of tricyclic antidepressants, in terms of inhibiting noradrenaline or 5-HT reuptake, can be assessed by estimation of catechol-O-methyltransferase metabolites, the levels of which will predominate over the normal MAO metabolites. The role of the serotonergic system, in general, and the 5-HT \textsubscript{2} receptor activity, in particular, in the genesis of endogenous depression, appears to be a major factor. Most anti-depressants appear to share the ability to increase 5-HT neurotransmission and 5-HT \textsubscript{2} receptor activity, by diverse mechanisms. There is compelling clinical evidence and experimental data in support of this hypothesis which indicates that depression may be a consequence of malfunctioning of the median raphe serotonergic system. The antidepressants which influence the serotonergic system include the reversible MAO A inhibitors (moclobemide, brofaromine, cimoxatone, befoxatone, toloxatone, etc.), selective serotonin reuptake inhibitors (fluoxetine, fluvoxamine, citalopram, sertraline, paroxetine, etc.), 5-HT\textsubscript{2} ligands (gepirone, ipsapirone, sunepitron, fleinoxan, etc.), 5-HT\textsubscript{2} receptor agonists (ritanserin, mianserin, amesergide) and TCAs with predominant effect on 5-HT reuptake (clomipramine, citalopramine, venlafaxine, duloxetine, milnacipran, etc.). Mirtazapine, which functions mainly as a central adrenergic alpha-2 receptor antagonist and increases noradrenaline release, is known to increase the release of 5-HT as well. These receptors are postulated to function as autoreceptors inhibiting 5-HT release from its neurones. Likewise, some of the so-called 'selective' noradrenaline reuptake inhibitor antidepressants, like maprotiline, bupropion, nortriptyline and desipramine, may not be entirely free from effects on the serotonergic system, including the 5-HT\textsubscript{2} receptors. However, some of the recently developed antidepressants do not appear to influence the monoaminergic reuptake system. Thus, rolipram appears to stimulate noradrenergic neurotransmission by, presynaptically, increasing noradrenaline synthesis and release, and, postsynaptically, by increasing cyclic-AMP concentration by inhibiting the enzyme phosphodiesterase. Similarly, acetyl-L-carnitine, which has a structural similarity to acetylcholine and stimulates muscarinic receptors, has been found to exhibit antidepressant effect in elderly depressed patients.

The major problem in evaluating an antidepressant in the laboratory is choosing the appropriate tests in which the new drug under investigation has to pass in order to be clinically evaluated at a later stage. All the tests mentioned above have been shown to have reliable predictive and face validity inasmuch that they are capable of identifying a variety of antidepressants acting via diverse mechanisms. However, each one of these tests have also been subjected to criticism because of the 'false positives' and 'false negatives' encountered by different laboratories. It has been shown that anticholinergic and antihistaminergic drugs exhibited antidepressant activity in a variety of animal models of depression, whereas opiates and neuroleptics appeared to accentuate the parameters used to assess depression. Ipsapirone, a clinically effective antidepressant, is
reported to be inactive in several animal models of depression, including the chronic stress paradigm. Three of the most popular methods used to screen antidepressant activity have been subjected to extensive scrutiny and the results are a mass of confusion. Whereas, the Porsolt's, learned helplessness and chronic mild unpredictable stress tests, have been effective in delineating newer antidepressants, there are reports that they are of relatively low validity, particularly when used to screen atypical antidepressants.

Depression has a number of temporal features, including spontaneous recovery, the periodicity of recurrent unipolar depression, and the alternation of depressive and manic phases of bipolar depression. These aspects remain relatively unresearched in animal models. However, although more documentation is required to assess the validity and reliability of animal models of depression, both in terms of the behavioural and neurochemical mechanisms involved, there is no doubt that the Porsolt's test, and later the learned helplessness test, were breakthroughs which supplanted the earlier used reserpine reversal test. These tests were simple and predictive, although lacking construct validity. The introduction of the chronic mild stress models was an improvement over these tests. However, despite the observation that this test may have predictive validity, its utility in antidepressant screening is limited by the chronically and, consequently, the time factor involved in the screening. While discussing the predictive validity and robustness criterion of animal models of depression, it has been commented that none of the existing models in use fulfill the expectations of the neurobiologist, in terms of predictive, construct or face validity. The answer to this vexing problem is to search for pharmacological properties shared by antidepressants belonging to different chemical classes and acting via diverse neurochemical mechanisms. The utility of such common denominators can serve as an acceptable approach in evaluating antidepressant activity. It has been suggested that investigators should be more modest in advocating the utility of existing animal models of depression and realize their shortcomings. In addition to their role in discovery of newer and more clinically effective antidepressants, animal models of depression are useful for providing insights into the neurobiology and pathophysiology of depression. However, the data derived from animal models are likely to be of value only to the extent that the models are valid, including consideration of predictive validity (which concerns primarily the correspondence between drug actions in the model and in the clinic), face validity (phenomenological similarities between the model and the clinical disorder) and construct validity (a sound theoretical rationale for using the model). The basic desirable features in an animal model are that the model should respond appropriately to standard antidepressant drugs, should employ physiologically realistic depression simulating conditions and should model a core symptom of the clinical disorder. Unfortunately, none of the existing models perform well against all three sets of validating criteria, including the widely used Porsolt's, learned helplessness and chronic stress tests. The acceptability of these, and the other tests used to evaluate antidepressants, is based on a reasonable pharmacological profile, with relatively few false positive and false negative. In the absence of a universally acceptable animal model, it is advisable to use a battery of tests in screening for putative antidepressant activity.

The choice of methods in preclinical screening of putative antidepressants is a difficult one, given the variety of methods available and the various ways in which the investigative drugs may function as an antidepressant. In our own experience, with the limitation of resources, the first preclinical indication of antidepressant activity should come from reversal of the reserpine syndrome. When a drug passes this initial test, the subsequent methods which are employed are the behavioural despair and learned helplessness tests. Later, potentiation of 5-HTP, I-DOPA, amphetamine and yohimbine actions are used to corroborate the earlier results and to get an indication of the neurotransmitter likely to be involved. Finally, the chronic unpredictable footshock method is used to provide conclusive data. The final proof will, however, come from clinical testing using well established parameters (HAM-D, MADRS, CGI severity).

References