Rodent Antidepressant Models in Neuropsychopharmacology: A Laboratory Perspective

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ABSTRACT:
Depressive disorder is a common illness that affects millions of people worldwide today becoming a leading cause for morbidity and mortality. Drugs with antidepressant properties in patients with severe depression also have various behavioural and neurochemical effects in animals. This has given rise to numerous animal models that have been suggested to be valid for research into the neurobiology of depression and the neurochemical mechanisms of the antidepressant drugs. The present review reflects on currently available animal models, discusses their potential to unravel the neurobiological underpinnings of depression and highlights their impact on the development of novel therapeutic strategies, along with the advantages and disadvantages of the models from laboratory perspective. The number of validated animal models for affective disorders is large and still growing. For the animal model of depression, the relevance, reliability and reproducibility in laboratories need to be focused, apart from ensuring good constructive, face and predictive validity. Whilst animal modelling remains a potentially important approach towards understanding neurochemical neurobiological mechanisms in depression, we need to tackle the dearth of reliable clinical science that should assist model development.

KEYWORDS: Antidepressant models, behavioural models, empirical models, neuropsychopharmacology, validity of animal models.

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1. INTRODUCTION:

Depressive disorder is a common illness that affects millions of people worldwide today. It is a leading cause of morbidity and mortality with a lifetime prevalence of about 15–20% and the patients often suffer from symptoms such as depressed mood, sleep disturbances and suicidal ideation\(^1,2\). Depressive episodes can be precipitated in some individuals by traumatic life events in childhood or adulthood and several animal models of depression have been generated accordingly. Existing animal models of human disease have proven of substantial value in elucidating basic pathophysiological mechanisms and in developing novel treatments. However, modelling human mental disorders in experimental animals is laden with complications as the animal models generally lack both clinical and scientific credibility and the same have thus, so far, failed to measure up to the complexity and heterogeneity of the clinical states labelled ‘depression’. Consequently, much of the neuroscience of animal modelling is framed around physiological and neurobiological phenomena that may be of relevance to only a minority of patients.

Also, the environment plays an important role in determining which behaviours a given species is predisposed to display based on lighting conditions and familiarity of experimental arena, social experience and familiarity of partner(s)\(^3,4\), territorial advantage\(^5,6\) and hierarchical position within a closed social group\(^7,8\), all the factors affect the social behaviour of rodents\(^9\).
Due to all the variables, it is exceedingly difficult to envision an animal model that perfectly recapitulates the symptoms of depression in human patients. However, depression, as other mental disorders, constitutes of intermediate or so-called endophenotypes that can be reproduced independently and evaluated in animals, including physiological, endocrinological and neuroanatomical alterations as well as behavioural traits. The minimal requirements for a valid animal model of depression have been proposed previously, which suggested that the model should be (i) reasonably analogous to the human disorder in its symptomatology (face validity), (ii) Cause behavioural changes that can be monitored objectively, (iii) Produce behavioural changes that are reversed by the same treatment modalities that are effective in humans (predictive validity) and (iv) Should be reproducible between investigators. The aetiological validity of a model refers to the similarity between the trigger that is used to precipitate neurobehavioural abnormalities in animals and suspected aetiological factors of human depression. Face validity is used similarly, to describe superficial likenesses between symptoms of human depression and those induced in animals. Predictive validity refers to the ability of an animal model to predict the therapeutic efficacy of antidepressant treatments. While, the constructive validity refers to the internal mechanism or state that underlies the depression. Based on the same, numerous behavioural paradigms have been established to elucidate face, predictive and construct validity of animal models of depression in various species (e.g. hamsters, voles, tree shrews, primates); but preclinical psychiatric research has clearly favoured the rat as animal model of choice until the advent and propagation of transgenic and gene targeting technologies in mice. Increasing efforts have been dedicated to the development of appropriate and valid rodent models to mimic major depression in humans; however, the extrapolation of the results to humans obtained with such models was taken cautiously and the validity of the models was under close scrutiny.

So far, no depression-like syndrome that fully recapitulates the human syndrome has been established in rodents. Most investigators have relied instead on combinations of environmental triggers and neurobehavioural endpoints in laboratory animals to screen for antidepressant drugs or to model specific symptoms of depression. In addition, several minor variations have been applied to each model; each of them having advantages and disadvantages.

Besides, all clinically useful antidepressant drugs (also called thymoleptics) potentiate, either directly or indirectly, the actions of norepinephrine, dopamine, and/or serotonin in the brain. This, along with other evidence, led to the biogenic amine theory which proposes that depression is due to a deficiency of monoamines such as norepinephrine and serotonin at certain key sites in the brain. The amine theory of depression was probably overly simplistic, since it is now known that the antidepressant
drugs, particularly the tricyclic antidepressants, affect many biological systems in addition to neurotransmitter uptake and it is not known which of these neurochemical systems is most responsible for the antidepressant activity; though the drugs with antidepressant properties in patients with severe depression also have various behavioural and neurochemical effects in animals. This has given rise to various animal models that have been suggested to be valid for research into the neurobiology of depression and the neurochemical mechanisms of the antidepressants. The data is summarized in the table 1.

**Table 1: Summary of major behavioural paradigms used to assess certain depression-like phenotypes in rodents, their validity, advantages and disadvantage**

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Paradigm</th>
<th>Validity</th>
<th>Advantage</th>
<th>Disadvantage</th>
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</thead>
<tbody>
<tr>
<td>1a.</td>
<td>Forced swim test (FST)</td>
<td>Predictive</td>
<td>-Sensitive to antidepressants.</td>
<td>-Sensitive to acute treatment only.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Easy to perform.</td>
<td>-Validity for non-monoamine antidepressants uncertain.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>-High reproducibility.</td>
<td>-Risk of hypothermia.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>-Low cost.</td>
<td>-Not specific for SSRI.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-High throughput.</td>
<td></td>
</tr>
<tr>
<td>1b.</td>
<td>Modified FST</td>
<td>Predictive</td>
<td>-Sensitive to antidepressant.</td>
<td>-Sensitive to acute treatment only.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Easy to perform</td>
<td>-Validity for non-monoamine antidepressants uncertain.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Can detect SSRI</td>
<td>-Risk of hypothermia.</td>
</tr>
<tr>
<td>2a.</td>
<td>Tail suspension test (TST)</td>
<td>Predictive</td>
<td>-Sensitive to antidepressant.</td>
<td>-Sensitive to acute treatment only and specificity is questionable.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Easy to perform</td>
<td>-Requires very strong stressors.</td>
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<td></td>
<td></td>
<td>-High reproducibility.</td>
<td>-Time consuming.</td>
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<td></td>
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<td></td>
<td>-Low cost.</td>
<td>-Ethical restrictions.</td>
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<td>-High throughput.</td>
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<tr>
<td>2b.</td>
<td>Modified TST</td>
<td>Predictive</td>
<td>-Sensitive to antidepressant.</td>
<td>-Not applicable in rats.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Easy to perform</td>
<td>-Applicable only in certain mouse strains.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>-High reproducibility.</td>
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<td>-Low cost.</td>
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<td>-High throughput.</td>
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<tr>
<td>3.</td>
<td>Learned helplessness (LH)</td>
<td>Predictive</td>
<td>-Good predictive validity including alternation in HPA axis activity and REM sleep characteristic of depression.</td>
<td>-Sensitive to some antidepressants.</td>
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<td>-Sensitive to acute treatment only and specificity is questionable.</td>
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<td>-Ethical restrictions.</td>
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|   | Chronic unpredictable stress (CUS) \(^{38, 39}\) | 4. | Predictive Face Construct | -Pharmacologically sensitive.  
-Respond to wide variety of antidepressants.  
-Few false negatives and positives.  
For example, anticholinergics and antihistaminics. | -Sensitive to chronic antidepressants.  
-Poor reliability and reproducibility.  
-Only some antidepressants are effective.  
-Labor intensive. |   |
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<tr>
<td>5.</td>
<td>Reward-based Sucrose preference and Intracranial self-stimulation (^{50})</td>
<td>Predictive Face Construct</td>
<td>Responds to chronic antidepressant treatment</td>
<td>The method is not specific</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Reserpine reversal (^{12, 55})</td>
<td>Predictive Face</td>
<td>Respond to tricyclic antidepressants and MAO inhibitors</td>
<td>Newer antidepressants are ineffective like mianserin and trazodone</td>
<td></td>
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</tbody>
</table>
| 7. | Olfactory bulbectomy \(^{66}\) | Face | Specific for all antidepressants amitriptyline, mianserin and viloxazine | -Some antidepressants show effects only after subchronic treatment  
-Effects are variable for different antidepressants |   |

### ANIMAL TESTS AND MODELS OF DEPRESSION

#### 2.1. FORCED SWIM TEST

A widely used classic model for antidepressant activity is the forced swim test (FST) as described earlier \(^{19, 20}\), one of the despair-based models which were proposed to have relatively good predictive validity for monoamine-based antidepressants \(^{12}\).

![Figure 1- Forced swim test in rats (Porsolt et al., 1978)](image-url)
The model is based on the premise that, when rats or mice are forced to swim in narrow cylindrical apparatus from there is no escape, after an initial period of vigorous attempt to escape, they adapt a characteristic immobile posture in which they remain floating in water making only those movements necessary to keep their head above the water level. The immobility exhibited by rodents reflects the state of “despair” as they resign themselves to the experimental conditions, which is taken as an index of depression.

![Figure 2- Chronic fatigue test (Kaur et al., 2000)](image)

In rats, drugs are given in two to three doses between the initial drug-free swimming session and the second swim that follows 24 hours later. On the other hand, in mice, it is sufficient to study the effect of drugs compared to vehicle given before the single swimming session. In both the species, clinically active antidepressants reduced the time spent immobile. The FST model, besides being simple and inexpensive, is very reliable across laboratories, have high specificity in detecting novel drugs and selectively sensitive to antidepressant treatments. The model was found to reduce immobility in tricyclic antidepressants, monoamine oxidase inhibitors and atypical antidepressants as well as electroconvulsive shocks. It was reported that psychostimulants reduce the immobility too; but in contrast to antidepressants, they caused marked motor stimulation. The false positives can be induced by opiates and antihistamines.

Drugs which increase synaptic nor adrenaline content were reported to reduce the immobility time in rats reflecting the activity of central catecholamine system; however, it was not clear which adrenocepter mediates this effect, since β-stimulant were ineffective tin the studies. As clonidine and yohimbine, agonist and antagonists of α2 adrenergic receptors, respectively, reduced immobility time and the α1-adrenoceptor stimulant, phenylehrine, reduced immobility time in the same studies but increases motor activity; it was found difficult to ascertain whether the effect could be attributed to this property. Plus, neither α1 nor β adrenergic antagonists were found to be active in the studies.
The effects on serotonergic drugs were found to be uncertain. If, on one hand, clomipramine, femofexine, fluoxetine and quzapine were shown to reduce immobility time, the drugs like citalopram including others did not reduced it over 20 percent\textsuperscript{21, 23, 25}. Also, drugs like 5-hydroxytryptophane and paroxetine were found to be ineffective, which were tested only after single dosing\textsuperscript{26}. Serotonergic antagonists had no effect in the studies on similar line\textsuperscript{21, 23, 25}.

Some experiments also have been carried out with drugs interfering with cholinergic transmission wherein it was found that anticholinergics reduced immobility time, and cholinomimetics increased it\textsuperscript{22, 27, 28}. Also, Several GABA-ergic drugs like sodium valproate and musimol were found to reduce the immobility time\textsuperscript{29}.

All dopamine mimetic substances were found to reduce immobility time and dopamine antagonists were either inactive or increased the immobility time in FST\textsuperscript{20, 23, 27, 29, 30}. The extensive data collected on the modified rat FST suggested the role of serotonergic antidepressants, in inducing distinct behavioural profile in the FST compared to antidepressants that act through catecholamine mechanisms\textsuperscript{31}, though their ability to detect non-monoamine-based antidepressants has been scrutinized and questioned\textsuperscript{30}.

Also, the method needed modification to be able to detect drugs that have their primary molecular mechanism of action selectively on the serotonergic neurons. Recently, some procedural modifications introduced in FST have enabled SSRI-induced behavioural responses to be measured\textsuperscript{30}.

The modified rat FST changed several of the testing conditions and scoring procedures used by the original FST procedure. A clear round cylinder of at least 20 cm diameter has been used with the modified FST and the water depth was increased from traditional depths of 15–18 to 30 cm. The modification of the FST also involved the development of a more complete description of the active behaviour of rats in the cylinder consisting of swimming and climbing or struggling; a multiple targeted behaviours which can be scored from videotapes instead of real-time scoring. Highly depressed genetic models (wistarkyto rats) showed recovery (by reduced immobility and increased climbing behaviour) and reduced Nor-1mRNA and GR mRNA transcripts, with the use of tricyclic antidepressants\textsuperscript{32}.

Selective serotonin reuptake inhibitors (SSRIs), currently the most widely prescribed antidepressants, were not reliably found to reduce immobility\textsuperscript{2}. It has been established in many laboratories that drugs acting primarily on noradrenergic neurons significantly reduce immobility and increase climbing/struggling, whereas SSRIs, as measured either by time-sampling of behaviour or separate real-time measurements of recorded behaviours, exert, in statistical terms, their most significant effect on swimming\textsuperscript{2}. FST provides a tool to probe the role of various neurochemical circuits and receptor
subtypes involved in the effects of antidepressants using a model relevant to stress-evoked depressive behaviour 33.

2.2. TAIL SUSPENSION TEST

Another despair based model is ‘Tail suspension Test’ (TST), described as a dry-land version of the FST 32. The test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, develop an immobile posture. In the test described, male, mice (Balb-cJ) were hung upside down by a wire, thread or by an adhesive tap (20 mm form the tip of the tail) 50 cm above the ground.

![Figure 3-Tail suspension test](Steru et al., 1985)

After an initial vigorous movement to escape the awkward situation, it assumed an immobile posture and the period of immobility during last 4 minutes of total 6 minutes duration were noted. Mice provided better results than rats and the test is simple, reliable and rapid for screening antidepressants and is able to separate locomotor stimulant dose from antidepressant dose. Another advantage is that, this test does not induce hypothermia that results from immersion of animal in to water in forced swimming test. Plus, it provides precise objective measurement of duration of immobility and is more sensitive to lower doses of drugs with clear dose effect relationships. An obvious advantage of this test is its ability to detect a broad spectrum of antidepressants irrespective of their underlying mechanism; it is inexpensive and unsophisticated, methodologically and easy amenable to automation. Automation also enables the assessment of additional parameters such as power of movements34, 35. Variety of antidepressants were found to reduces the duration of immobility and so were the psycho stimulants and atropine; in contrast to which diazepam was found to increase the same 33.
2.3. CHRONIC FATIGUE TEST

Another form of modified behaviour despair test is chronic fatigue test, in which FST is done continuously in rats or mice where they are forced to swim individually in glass jar for 7 days which produce depression and fatigue resembling chronic fatigue syndrome (CFS). CFS is a heterogeneous disorder of unknown etiology characterized by fatigue, neuropsychiatry symptoms and related somatic complaints. Chronic stress is a risk factor for development of many psychopharmacological conditions in humans, including major depression and anxiety disorders. It does play a role in the aetiology of melancholia and continual presence of it during antidepressant therapy would usually be the norm. The reversal of an established behavioural deficit during the continued presence of the stressor is an important feature of this model.

To understand the neurobiological mechanism underlying depression, cognitive and emotional consequences chronic stress, it is very necessary to employ an animal model that exhibit similar effects. Repeated presentation of the same stressor usually leads to adaptation. However, adaptation can be prevented by presenting a variety of stressors in an unpredictable sequence which can produce a behavioural state characteristic of depression in rodents.

Based on this, previous studies have shown that three weeks of exposure to electric shocks, immersion in cold water, immobilization, reversal of the light/dark cycle followed by session of exposure to loud noises, bright lights and variety of other stressors when followed immediately by an open field test, showed increase in open field activity and the effect, which was not observed in chronically stressed animals.

Figure 4-Open field activity in mice (Bhattacharya, 1993)
The model has a great many positive features and is probably the most valid animal model of depression currently available.

One study has investigated the behavioural parallels between adaptation to stress and antidepressant treatments using the FST. Electroacupuncture also has potential antidepressant-like effect on Chronic unpredictable stress-induced depression model in rats, which was mediated by affecting the glial atrophy in the hippocampus. Chronic unpredictable stress also induces a reduction in the Brain derived neurotropic factor gene expression with a concomitant diminution of serum zinc concentration. Restraint stress given repeatedly for 11 days significantly reduced immobility on this test while, a single application of stress had no effect. The reduction in immobility produced by repeated restraint was quantitatively similar to that produced by repeated administration of desmethylimipramine. These results confirmed previous findings of similarities in the behavioural and neurochemical response to chronic stress and chronic antidepressant treatment, where it was found that, antidepressants and stress when administered chronically; they reduce the density of beta adrenoceptors in various region of rat brain, which was accompanied by decrease in noradrenaline-sensitive adenylate cyclase activity, although the relationship between stress and depression remained incompletely understood. Stress models like chronic unpredictable stress and chronic mild stress produced neurophathological changes in rat hippocampus and behavioural paradigms in these models are in mainly due to stress induced reduction of calcium binding neurons.

A variety of antidepressant drugs were found to prevent the effect of chronic stress excepting MAOI tranylcypromine. In contrast, some psychotropic drugs lacking antidepressant activity failed to prevent the effect of stress. In addition to causing changes in open field activity, chronic stress also increased plasma corticosteroid levels. This effect showed the same spectrum of pharmacological sensitivity, with the exception that an anticholinergic was also effective. The chronic stress model has been utilized little because of the levels of severity employed raise serious ethical problems. Also, a major drawback is that the model has proved extremely difficult to implement reliably and reproducibly in laboratories.

2.4. LEARNED HELPLESSNESS

The learned helplessness paradigm is one model based on the observation that animals exposed to uncontrollable stress (usually electric shocks) in one situation, subsequently fail to escape shock in a different situation when escape is possible.
This model is reported to have a good predictive validity including alterations in hypophyseal-pituitary axis (HPA) activity and rapid eye movement (REM) sleep characteristic of depression and can be used as an additional screening procedure but the model is time consuming and its specificity is questionable. Other problems with this model are that the changes persisted for only a couple of days, reducing ease of method use and the need to repeatedly administer shocks that has contributed to its unfavourable image, even unacceptable by ethics committees in some countries.

2.5. REWARD BASED MODELS

In contrast to the extensive array of drugs correctly classified in chronic mild stress experiments, very few false positives or false negatives have been reported for reward based models\(^48, 49\). In the studies mentioned, antidepressant treatment were found to have no effect on sucrose consumption or intracranial self stimulation (ICSS) threshold in non stressed animals but following the reduction of sucrose intake by stress, normal behaviour was gradually restored by chronic treatment (two to five weeks) with a wide variety of antidepressants, including tricyclic antidepressants (TCAs), SSRIs, a specific noradrenaline reuptake inhibitor (maprotiline), monoamine oxidase-A inhibitors (MAO-A inhibitors), atypical antidepressants such as mianserin, buspirone, sulpride and also properties of food, as assessed in above paradigm \(^51\). Stress models appear to have greater aetiological validity compared with those that rely on brain lesions, immune stimulations or monoaminergic depletion some agents of uncertain antidepressant status, such as antihistaminic and anticholinergic drugs. On the other hand, the ineffective drugs include the anxiolytic chlordiazepoxide, various neuroleptics, amphetamine, and morphine. Fluoxetine, maprotiline and mianserin (but not chlordiazepoxides) were also found to restore the rewarding, which are not common aetiological factors in human depression. Although some equate
aetiological validity with construct validity; For example, evidence of reduced reward in animal models might be related in terms of underlying mechanisms to symptoms of anhedonia in humans with depression. Studies of the neural basis of the chronic mild stress-induced anhedonia have focused primarily on the mesolimbic DA system. The behavioural changes in animals subjected to this model are accompanied by a decrease in D2/D3-receptor binding and D2-mRNA expression in the nucleus accumbens, and a pronounced functional subsensitivity to the rewarding and locomotor stimulant effects of the D2/D3 receptor agonist quinpirole, administered systemically or within the nucleus accumbens. All of these effects were also found to be reversed by chronic antidepressant treatment.

The earliest pharmacological models of antidepressant-like activity had significant impact on establishing the monoamine theory of depression, which assumes that an elevation of serotonin and norepinephrine levels will improve depressive symptomatology.

### 2.6. RESERPINE REVERSAL

The reserpine model is based on the capability of antidepressants to reverse the inhibitory effects of reserpine on motility, body temperature and ptosis antagonism in rats and mice as reserpine, an antihypertensive and antipsychotic drug, is capable of non-selectively depleting brain monoamines and thereby induces a syndrome of locomotor hypomotility and reduced body temperature in rodents. A similar approach is underlying the 5-hydroxytryptophan (5-HTP)-induced behavioural syndrome.

To identify compounds that enhance synaptic concentration of serotonin, the test detects elevated levels of serotonin by measuring the potency to further increase the behavioural syndrome induced by administration of 5-HTP, which is the metabolic precursor of 5-HT. The test provides a rapid and accurate index of selective serotonin reuptake inhibitor (SSRI) potency in vivo. Animals with lower levels of 5-HT1A autoreceptors are more resilient to repeated exposures of forced swim stress compared to animals with higher levels of autoreceptors. These models offer good predictive validity in terms of monoamine-based antidepressant activity; albeit they do not model core symptoms of depression.

Concurrent with these studies, other groups have focused on neurochemical and physiological alterations that might account for the antidepressant sensitive behavioural alterations. Much interest has been placed on the serotonergic system with a 5-HT hyperinnervation of the frontal cortex and stressor-induced alterations in 5-HT-mediated activity observed subsequent to bulbectomy. Furthermore, increased striatal glutamate release during novelty exposure-induced hyperactivity in the test has postulated a modulatory role of glutamate on the antidepressant-sensitive response. Also the AMPA receptor activation may play an important role in both the rapid and sustained antidepressant
like effects of ketamine in animal models of depression, like LH and TST, but its antagonist NBQX did not reverse the effect. Increase in the concentrations of the neuropeptides (or their encoding genes) corticotropin-releasing factor, thyrotrophin-releasing factor, somatostatin and neuropeptide Y, which might play a role in mediating the antidepressant-sensitive behaviours, have also been established.

2.7. OLFACTORY BULBECTOMY
Rats anesthetized with tribromoethanol and skull exposure with holes drilled anterior to bregma and either side of the midline at a point corresponding to the posterior margin of the orbit of the eye. The olfactory bulbs were removed by suction, the holes were filled with haemostatic sponge in order to control the bleeding and the scalp was sutured Sham-operated animals received the same surgical treatment, but the bulbs were left intact and further subjected to open field and passive avoidance test. A variety of behavioural changes, like irritability, hyperactivity and an elevation of circulating levels of plasma corticosteroids; as a result of their hyperactivity. All of these changes can be reversed by antidepressant drugs.

Imaging studies demonstrated alterations in signal intensities in cortical, hippocampal, caudate and amygdaloid regions in olfactory bulbectomized animals compared with sham-operated controls. In addition, from the ventricular enlargement evident in bulbectomized animals, it has been suggested that these structural changes correlate somewhat with those seen in depressed patients. Over the years, several modifications of the existing models and new neurochemical rodent models have been developed to explain depressive disorders.

2.8. GENETIC MODELS
Of late, the power of genetic approaches has led to a significant shift in the concept of genetic models of depression. There are several genetic and non-genetic aetiologically sources of early origin of individuality which are used to identify factors underlying predisposition to depression have been summarised previously. The more traditional approach of selective breeding to provide different behavioural phenotypes has been supplemented by genetically modified mice. As the first genetic model of depression Flinders Sensitive line (FSL) and Flinders Resistant Line (FRL) were selected for their differential hypothermic responses to an anticholinesterase agent. FSL rat is more susceptible in developing aberrant behaviors related to depression followed metabolic stress, induced by high fat diet, as compared to FRL rat. Animals selectively bred for their differential susceptibilities to stress-induced changes in swim-test activity and strains with a high immobility in the swim-test such as the
Fawn- Hooded rat and Wistar-Kyoto rats as genetic animal models for depression\textsuperscript{72, 73} were described. Genome wide microarray analyses in chronically stressed wistar-kyo rats identified the presence of expressed genes in hippocampus and amygdala suggesting some new treatment and unexplored molecular mechanism for major depression and proved that molecular underpinnings differ from endogenous depression in animals\textsuperscript{74}. The same could be explored further to target and evaluate novel antidepressant agents which could prove to be successful in treating depression resistant to conventional therapy. The FSL rats exhibited a high predictive validity and exhibits changes in brain that were found to be consistent with the cholinergic, serotonergic, dopaminergic, NPY, and circadian rhythm models but not the noradrenergic, HPA axis or GABA-ergic models of depression\textsuperscript{69}. An attempt was also made to show the relationship between circadian rhythm and depression, by using stress paradigms, either to naïve animals or prenatal, breeding for specific phenotypes, brain lesions and genetic\textsuperscript{75}.

Refined molecular technologies and the creation of genetically engineered mice have allowed to specifically target individual genes involved in regulation of corticotropin releasing factor (CRF) system elements (e.g. CRF and CRF-related peptides, their receptors, binding protein), as described earlier\textsuperscript{76}. Transgenic mice overexpressing CRF (genetic C57/B6!SJL) exhibited prominent endocrine abnormalities involving the HPA system, such as high plasma levels of ACTH and corticosterone and displayed physical changes similar to the stigmata seen in patients with cushing’s syndrome, such as excess fat accumulation, muscle atrophy, thin skin, and hair loss\textsuperscript{77}.

Rats have been selectively bred for susceptibility to learned helplessness\textsuperscript{78, 79}. Apart from high and low level of immobility in mice in the FST\textsuperscript{72}, other genetic models are based on an underlying alteration in the function of both cholinergic and serotonergic neurotransmitter systems\textsuperscript{80, 81}.

A mouse model has also been derived from animals bred for spontaneous high or low immobility scores in the TST (H/Rouen mice ‘depressed’ or ‘helpless mice’)\textsuperscript{82}. Many such stress models of depression by forming genetically vulnerable strains have been described based on the plausible interaction between stress and genetic vulnerability\textsuperscript{83, 84}.

2. CONCLUSION AND FUTURE PROSPECTS:
From discussion above, it is apparent that there is a significant overlap between the functional abnormalities in rodent models and those changes that have been reported to occur in the patient with major depression. Several minor variations have been applied to each model. All models consist of a manipulation and at least one dependent variable, the choice of which is based on the aspect of
depression that one wishes to model. The choice and design of a dependent measure is not always easy due to the fact that our knowledge of the etiology of depression is still limited and based largely on inferences drawn from the presumed modes of action of clinically available antidepressant treatments. In addition, despite every possible attempt to standardize experimental conditions at different laboratories; important inter-laboratory differences, including significant strain-effect interactions, still emerge and it seems advisable to ensure that the animals belong to the specific species and are in bred to avoid the variability of the results. Many avenues are opening up for researching animal models of depression. New research is progressing on the role of nitric oxide (NO) -mediated neurotransmission in the dorsal raphe nucleus in producing significant and complex motor and emotional effects in animal models of anxiety and depression and on the effect of caloric restriction induced neurochemical and behavioural changes in rats consistent with depression. Knockout strategies have opened up an entire new avenue for selection of drug targets in depression.

As the current models are refined continuously to reveal the therapeutic potential of a broad range of compounds, the old rule of thumb is to ensure the use of same bred animals in the experiments for uniformity of the results, use of oral graded dose (Intraperitoneal, in case of new drugs), subchronic administration (3-5 days) in case of plant extracts and avoiding false negatives or false positives at the same time using a battery of tests in screening antidepressants in laboratories which need to be complemented by the corroborative biochemical paradigms. Also, for nocturnal animals like rats and mice, determination of the effect of psychotropic drugs on natural action patterns of behaviour should employ observations during the dark phase of the light-dark cycle. A strategy of inducing the depressive state and measuring the same in rat, using neural circuits (as both the independent and major dependent variables) was achieved by repeated electrical stimulation of the brain (ESB) within the dorsal periaqueductal gray in brain. Mice when exposed to 22 hr artificial day light produced spectrum of behaviour and endocrine symptoms which can be a simple and new method for study of antidepressant. An approach was made to study gene and stress interactions by modeling the behavioral and neural consequences of stress genetically in C57BL/6J mice, increased light compartment exploration, reduced body weight and sensitized the corticosterone response to swim stress.

Despite the fact that none of the presently available animal models is able to model all aspects of depression and most likely never will; existing paradigms have proven extremely useful not only in the identification and improvement of antidepressant substances, but also in the validation of neurobiological concepts. To strengthen paradigms modelling disease etiology to improve their reliability and to develop novel tests that will allow to pick up classes of antidepressants beyond
monoamines pose major challenges for the future. A recent study showed that different types of environmental stress also enhance glutamate release/transmission in limbic/cortical areas and exert powerful structural effects, inducing dendritic remodeling, reduction of synapses and possibly volumetric reductions resembling those observed in depressed patients which can be newer targets for antidepressants drugs. To accomplish these goals it seem necessary to implement recent advances emerging from psychiatric genetics, non-invasive neuroimaging research and biomarker identification. As important an issue is the full incorporation of ethical 3R (Replacement, Reduction and Refinement) principle for reducing suffering and distress and maintaining optimum welfare in animals to ensure ethical and scientific validity to animal experimentation which might eventually result in improved methodologies over time.

3. REFERENCES


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