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REVIEW ARTICLE

A review on animal models for screening potential anti-stress agents

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Abstract Stress is a state of threatened homeostasis that produces different physiological as well as pathological changes depending on severity, type and duration of stress. The animal models are pivotal for understanding the pathophysiology of stress-induced behavioral alterations and development of effective therapy for its optimal management. A battery of models has been developed to simulate the clinical pain conditions with diverse etiology. An ideal animal model should be able to reproduce each of the aspects of stress response and should be able to mimic the natural progression of the disease. The present review describes the different types of acute and chronic stress models including immersion in cold water with no escape, cold environment isolation, immobilization/restraint-induced stress, cold-water restraint stress, electric foot shock-induced stress, forced swimming-induced stress, food-deprived activity stress, neonatal isolation-induced stress, predatory stress, day–night light change-induced stress, noise-induced stress, model of post-traumatic stress disorder and chronic unpredictable stress models.

Keywords Acute/chronic stress · Chronic unpredictable stress · Immobilization · Noise · Cold immersion

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Introduction

Stress is a state of threatened homeostasis that produces different physiological as well as pathological changes depending on severity, type and duration of stress [106]. Exposure to hostile conditions (i.e. stressors) results in a series of coordinated responses organized to enhance the probability of survival [46–48]. The physiological changes associated with stress are mobilization of energy to maintain brain and muscle function; sharpened and focused attention of the perceived threat, increased cerebral perfusion rates and local cerebral glucose utilization, enhanced cardiovascular output, respiration and redistribution of the blood flow and modulation of immune system [40, 46]. Prolonged stress has been documented to play an important role in depression and neurodegenerative disorders. Behavioral stress has also been recognized as a main risk factor for many diseases including cardiovascular, metabolic and neuropsychiatric diseases [95, 108] (Fig. 1). A correlation has been established between offensive subtype of social anxiety and personality disorders [96]. Furthermore, studies have shown that stress may enhance the progression of various diseases including colitis [90]. Stress begins with a stimulus of external or internal origin to the organism that activates the hypothalamic–pituitary–adrenal axis (HPA) and the sympathetic nervous system (SNS) [128]. This activation results in a compensatory physiological change or adaptation so that the organism can deal with the threat [83]. The hypothalamic–adrenal axis and SNS activation leads to generation of glucocorticoids [9] and catecholamine which together are called "stress hormones". These glucocorticoids inhibit HPA axis activity by feedback mechanism by binding to their receptors in the pituitary gland, hypothalamus and medial prefrontal cortex [27, 134]. Though stress and anxiety are not very much

different from one another, yet, at the same time they cannot be considered as same. Stress is a state of threatened homeostasis that triggers reactions in the body to cope with stress in the form of adaptation. The failure to do so leads to development of different psychological disorders and anxiety is one of the disorders like depression. Therefore, anxiolytic agents actually do not denote anti-stress agents.

Different animal models for stress have been developed (Fig. 2) and used frequently to evaluate the anti-stress activity of compounds of both natural and synthetic origin. Research associated with stress has focused on identifying novel therapeutic modalities and understanding the mechanism of stress response by employing appropriate animal model of stress. An ideal animal model should be able to reproduce each of the aspects of stress response and should be able to mimic the natural progression of the disease. Researchers have developed various acute and chronic models of stress including immersion in cold water, cold environment isolation, immobilization/restraint-induced stress, electric foot shock (EFS)-induced stress, forced swimming-induced stress, food-deprived activity stress, neonatal isolation-induced stress, predatory stress, day–night light change-induced stress, noise-induced stress and models of post-traumatic stress disorder. The present review describes these different physical and psychological based acute, chronic and chronic unpredictable stress models.

Animal models of stress

Physical stress models

Many of the physical models are based on fluctuation in body temperature. Acute change in temperature leads to stressful conditions by activation of temperature regulatory center in the hypothalamus and subsequently HPA axis. It leads to acute release of adrenocortical hormones in the blood stream responsible for acute stressful response [115]. It causes impairment in thermoregulatory capacities. So these animals are impaired in terminating the secretion of adrenocortical stress hormones, glucocorticoids, at the end of stress. This hormonal excess may be due to degenerative changes in a region of the brain which normally inhibits glucocorticoid release; the degeneration, in turn, is caused by cumulative exposure to glucocorticoids. A sharp decrease in temperature using either cold water or freezer has been used frequently to induce acute stress.

Immersion in cold water (ICW)

In this method, the rats are placed individually in a tank (25 × 35 × 40 cm) of cold water (depth 15.5 cm; temperature 15–20°C) for 15 min, where they either swim or remain in an upright position, keeping their heads above the water level [3, 6, 110, 120, 137]. A modification has

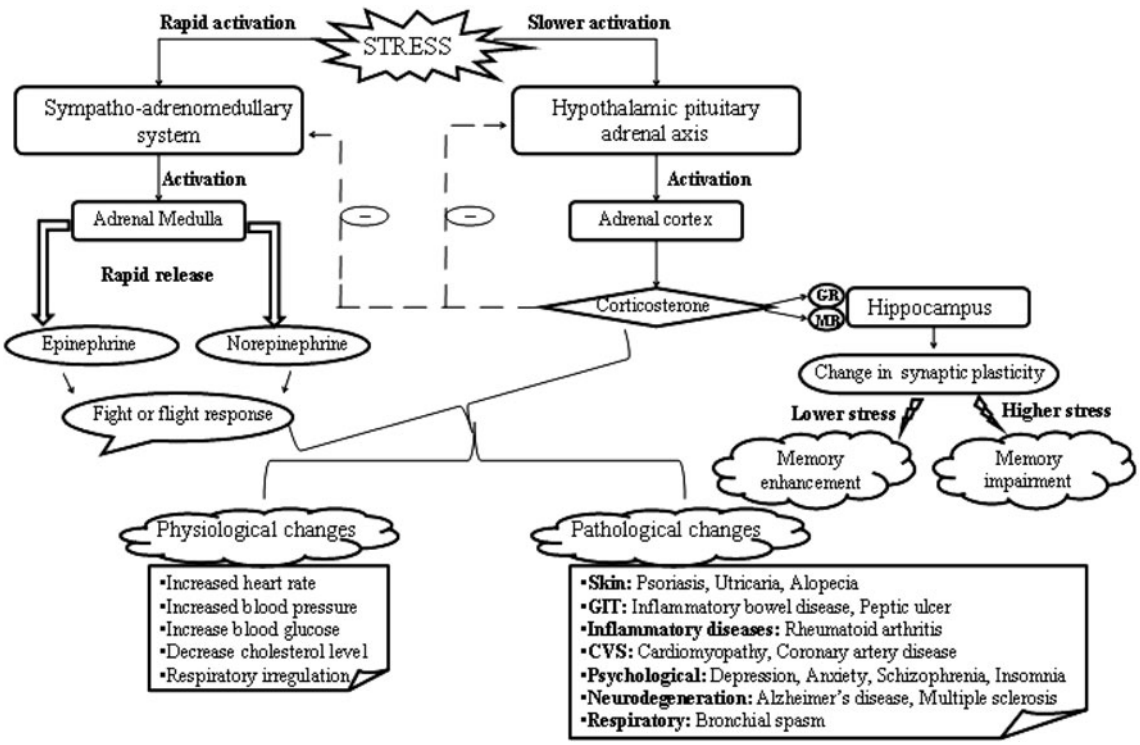

Fig. 1 Representative diagram of different physiological and pathological changes in the body in response to stressor. *Dashed line* negative feed mechanism; *GR* glucocorticoid receptors, *MR* mineralocorticoid receptors

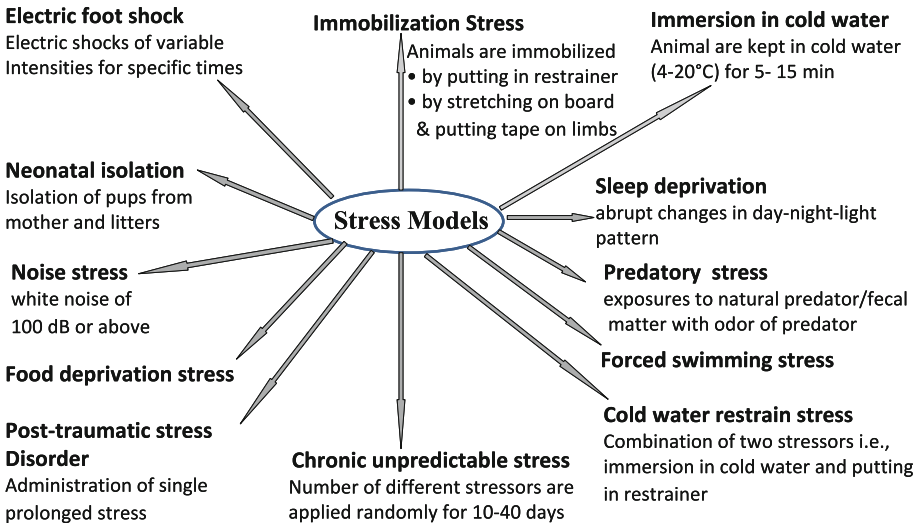

Fig. 2 A diagrammatic representation of different animal models employed for inducing acute, chronic and chronic unpredictable stress in different animals

been made in the method by subjecting the animals to cold-water immersion stress for 5 min at 4°C [76, 120] and this situation lasts for 15 min unless the rats sink. In that event, rats are removed before the cut-off time and are not included in the experiments. Stressors are applied, both acutely (5–15 min) and chronically (during 4, 12 and 20 days) at the onset of the light phase as well as at the onset of the dark phase of the light/dark cycle [7]. Immersion in cold water elicits a clear increase in plasmatic corticosterone levels [110], regardless of the time cycle of the stressors. For acute stress, rats are killed 30 min after the stress exposure. For chronic stress, animals are exposed to this stressor for 7–10 days and thereafter, the rats are killed 1 h after the last stress session. The major advantage of this type of stressor is that acute stress can be achieved in a relatively short period of time [56]. However, the major drawback of this model is that the body adapts to change in temperature on chronic exposure to low temperature and hence, stress response gets highly diminished with repeated episodes of stress [18, 102].

Cold environment isolation

In this method, rats are individually kept in a freezer with a temperature maintained at 4°C for 15 min once for acute stress and for 7–10 days to develop chronic stress [21, 68, 71, 103]. In a modified method, cold exposure in a small refrigerator set at 4 ± 2°C lasting for 30 min has been provided for inducing stress [130]. In another modification, the rats are exposed to cold stress by placing them in wire mesh cages in an open refrigerated compartment at −8°C for 4 h. Rats are exposed to this environment only once and their behavior is observed throughout the stress experiment along with body temperature monitoring [93]. This sharp fall in temperature leads to a sharp increase in the levels of adrenocorticoids culminating in the development of stress response [72, 123]. Unlike the ICW model, rats are prevented from drowning in cold water and hence, it is relatively safe model. However, it also suffers from same drawback of development of resistance/adaptation on chronic exposure.

Immobilization/restrain-induced stress

Immobilization has been used extensively as a stressor for the study of stress-related biological, biochemical and physiological responses in animals [15, 54, 73, 89]. Immobilization stress induces vascular oxidative stress by activating the angiotensin II/AT1 receptor signaling pathway, thereby provoking endothelial dysfunction which can contribute to the development of atherosclerosis and hypertension [22]. Immobilization stress is of two types: immobilization-induced stress with restrainer and immobilization-induced stress without restrainer. Restraint stress has been one of more commonly employed model for the induction of acute stress in rats [70] and this type of physical stress has been useful for studying stress-induced neurodegeneration and post-traumatic disorders [121]. Restraint stress has been induced by placing the rats individually in the 5.5-cm-diameter and 18-cm-long semi-cylindrical, acrylic restrainer with air holes for 3.5 h [55, 84, 87]. There have been variations among different models of this category with respect to difference in the size of restrainers and time interval of restraining the rats [26, 30]. This model has also been utilized to produce chronic stress by placing the rats in a 25 × 9 × 7 cm plastic bottle with a 1.5-cm-diameter hole at one far end for breathing, and fixing it with plaster tape on the outside to restrict the movement of animal. The animals are restrained for 1 h/day, 5 days a week for 40 days [24]. Mice have also

been employed as animal models and these are subjected to acute restraint stress by placing them individually in a well-ventilated polypropylene tube (40 mm in diameter and 90 mm in length) for 2 h [116]. Although the animal's range of movement is severely limited, the limbs are not secured and the animal remains within an enclosed area in restraint stress. Accordingly, scientists have reported that restraint stress is less intense a stressor than immobilization, and comparative studies using neural and endocrine measures have supported this assertion.

Another way is to keep the animal with its limbs stretched on a board and immobilized with adhesive tape. Movement of head is restricted by keeping the head in a metal loop coiled around the neck. The rats are kept immobilized for 150 min once to produce acute stress and for 7–10 days to produce chronic stress. Immobilization-induced stress can be produced by subjecting the animals to variable stress duration such as 1 h [109], 2 h [38, 39], 3 h [127, 138], 4 h [61] and 6 h [28, 70]. Immobilization is a complex stressor and includes physical as well as psychological dimensions. The struggling and associated muscular exertions that occur during immobilization represent an intense form of physical exercise. The major advantage of using immobilization as a model of stress is that it produces an inescapable physical and mental stress to which adaptation is seldom exhibited [54]. Immobilization-induced stress has been one of more commonly employed model for the induction of acute stress in rats [70, 122] and this type of physical stress is most useful for studying stress-induced neurodegeneration and post-traumatic disorders [1, 121] and is also one of the most useful models in the analysis of stress effects on the immune system [45].

Cold-water restraint stress

It is combination of both immobilization and cold stress. The combination of body-restraint procedure with exposure to the cold environment drastically increases the occurrence of gastric ulceration in a shorter period of time (3 h). Two different cold exposures have been employed to enhance body-restraint ulceration. One of two different cold exposure procedures involves the immobilization of the animal, generally in a supine position, and then placing the animal in a cold environment (5 ± 1°C) such as a refrigerator for 3 h [38, 81, 103]. The other procedure involves the restraining of animal in a cylindrical tube and then immersing vertically in cold water (22°C) for 1 h [59]. It has been shown that combination of these two stressors produces severe form of stress that mobilizes catecholaminergic systems in brain areas associated with behavioral responses to aversive stimuli as well as neuroendocrine responses of the HPA axis, including changes in corticotrophin-releasing factor (CRF), adrenocorticotrophin hormone (ACTH) plasma levels and adrenergic receptors in the pituitary [36]. It has been suggested that combinations of different stressors are better ulcerogenic stimuli when compared with each one alone. Some researchers have made modification in the method by increasing the duration to stress exposure up to three and a half hour [20].

EFS-induced stress

EFS of mild intensity has also been used as a stressor. Rodents are very susceptible even to mild shock and exhibit rapid stress response. Researchers have used EFS of varying degree to produce stressful conditions and hence to evaluate adaptogenic activity of various compounds. Stress by EFS is given by placing the rats individually in a chamber with an electrified floor. It has been reported that the stressor are considered clinically relevant on repeated administration. Accordingly, foot shock stressor is usually applied repeatedly. In one of the protocols, rats are administered EFS of 2 mA intensity of 10-s duration at the interval of 50 s for 2 h in a day for 14 days using EFS generator [136]. Earlier reports had shown development of EFS-induced analgesia in rats by applying inescapable and unsignaled shock of intensity of 2 mA, 0.2 Hz and 1-s duration for 30 min [51, 52]. In another protocol, stress has been produced in mice by applying EFS for 0.5 s duration after every 5 s for 30 min for five consecutive days. In this protocol mild current was applied, just sufficient to produce behavior changes in animals such as jumping or squeaking, and the current intensity was kept below 1 mA [139].

Various other modifications have been done in the EFS-induced stress by changing the current or duration of shock. Four unsignaled electric shocks (2 s, 0.8 mA, pulsed) are delivered at a variable inter-stimulus interval of 1 min in a box equipped with a grid floor consisting of 24 electrifiable steel rods and electrifiable walls [33]. In another type, rats receive unavoidable EFS with an intensity of 3 mA (50 Hz), duration of 200 ms and a frequency of 1 per second over a 5-min period [110]. Other models include administration of inescapable EFS for 60 min (0.15 mA shock, on a variable interval schedule with a mean inter-shock interval of 60 s) [126]; 90 V, 0.8 mA for 1 s randomly for 30 and 60 min in total [133]. For acute stress response, the rats are exposed once and killed after 15 min of stress. Chronic stress is also produced by repeating the same treatment for 7–10 days and rats are killed 1 h after the last stress session. The biggest advantage of this model is that it effectively produces high degree of stress in the animal. The major disadvantage of this model is the hazard of electric shock causing death of the animal and special caution that is required to perform this methodology.

Forced swimming induced stress

It is the tendency of the living being to escape or avoid a noxious stimuli/condition. If the animal is not able to escape the stressful stimuli or it feels threatened, the animal will show stress response. This principle is used for developing forced swimming model for inducing stress in laboratory animals. In order to produce swimming-induced stress, rats are made to swim in a cylinder (30 cm in diameter and filled to a height of 20 cm with 15 cm of space above the head of the rat) for a single session of 2-h duration for acute stress, or for one 2-h session a day for five consecutive days for chronic stress [37]. Some scientists have used forced swimming in warm (20°C) water for 3 min with the total session lasting for 1 h [58]. Other researchers have used forced swimming in cold water (4°C) in a container 15 cm in diameter and 20 cm in height with water filled to a depth of 11 cm for 3 min [119, 124]. Another modification made in the method is forced swimming in water at 22 ± 1°C [8]. Although forced swimming-induced stress is a highly safe model, adaptation to chronic swimming-induced stress has been reported and inter-strain differences between rats to forced swimming behavior have also been documented [6].

Food-deprived activity stress

Food-deprived activity stress has been defined as the condition in which rats were forced to run on a wire wheel while food consumption is restricted. Food-deprived activity stress gradually increases the hyperactivity on the running wheel and decreases food consumption [32]. Food-deprived activity stress reduces food consumption [32], changes the locomotor activity and induces hyperactivity of wheel running [94]. The animals are subjected to forced running on an activity wheel and are also subjected to an additional stress induced through food deprivation for 22.5 h/day and are permitted to take food and water for 1.5 h/day [10].

Psychological stress models

Repeated social defeat stress

Repeated social-defeat stress provides a more naturalistic model of stress characterized by aggressive interactions that are intense, unpredictable and inescapable. The social defeat model has been characterized by the physiological and behavior associated with anxiety and depression. Social defeat is considered an ecologically and ethologically relevant animal model of psychosocial stress that produces enduring behavioral and neurochemical sensitization in defeated individuals. Social defeat stress consisted of a brief aggressive confrontation between experimental intruder rats and aggressive resident rats. To induce social defeat stress, a mouse (the 'intruder') is transiently placed in the home cage of a resident male mouse (the 'aggressor') [53, 91, 98]. Before starting the social stress procedure, the resident male mouse is housed with a normal cycling female to enhance territorial behavior and aggressiveness and is followed by removal of females from the resident's cage. Thereafter, the intruder mouse is introduced for a 20-min trial and five such trials are given a day for 3–6 days. Alternatively, rats are exposed to social defeat once every 72 h over the course of 10 days (i.e., four stress exposures) [34]. The social defeat behavior is characterized by social defeat posture consisting of immobility; escape; crouching (four paws on ground, not orienting toward resident), and defensive upright stance (standing still and erect with forepaws extended).

Psychological stress with communication box

Ogawa and Kuwahara [99] described the development of psychological stress using communication box in mice and others have modified the method. In this method, the mice are placed in the 30 × 30 × 30 cm chamber with a grid floor composed of 1.5 mm stainless steel rods 7 mm apart from each other and divided into nine compartments of 10 × 10 × 30 cm with transparent plastic walls. A scrambled electric shock (2 mA, 0.2 Hz, 1 s duration) is delivered for 5 min through the floor grid. Plastic plates placed on the grids of five compartments prevent the animals from receiving direct electric current. However, the mice are exposed to psychological stress for 5 min by watching and hearing the struggle, jumping and vocalization of shocked animals in the adjacent compartments on application of electric shock [52].

Neonatal isolation stress (maternal deprivation induced stress)

Early life events have profound consequences on subsequent quality of life [75]. It has been shown that the early life stress of neonatal isolation in rats has immediate and enduring neural and behavioral effects [69]. Rearing rats in isolation post-weaning is an animal model of social deprivation that recapitulates features of limbic-based psychopathology in humans. Rodents reared in deprivation of social contact exhibit an abnormal behavioral phenotype that includes hyperlocomotion in response to a novel environment [49], altered habituation and disrupted exploratory behaviors [31, 129]. Brief isolation of an individual pup from the dam and litter, repeatedly, is an effective method to stimulate HPA axis without altering the growth in neonates [50] and adults [57]. Such effects

may reflect the stress-induced morphological changes in hippocampus and other brain regions [62, 66]. In fact, the hippocampus provides negative feedback regulation of the HPA axis and hence neonatal isolation-induced stress can represent the stress response that may lead to neurodegeneration at an early stage of life. This stress procedure is also useful in evaluating the effect of stress on cognition and memory development. In the neonatal isolation procedure, the litter of the inbred strain is removed from the cage on the second day after the birth, weighed and placed individually in an opaque plastic container (9 cm diameter and 8 cm deep) with no bedding for 1 h (between 0900 and 1200 hours) in a heated (30°C), humidity-controlled chamber with white noise to mask other pups' calls. The chamber has to be located in a room separate from animal colony facility. Containers are placed 20–30 cm apart [60, 62, 67]. After 1 h period the litters are placed back with their dams in home cage [64, 65]. This isolation procedure continues up to 8 days and hence it is used to induce chronic stress only. Neonatal isolation stress model has been used extensively to demonstrate the effect of early lifetime stress on vulnerability to addiction [63] and response to psycho stimulants by impairment of hippocampus-dependent context induced fear in adult male rats [66]. Neonatal isolation model has been used extensively to study the persistent changes in the dopamine levels resulting from neonatal stress [67].

Predatory stress

Direct encounter of an animal with its natural predator is one of the most stressful and anxiogenic event it can face and it leads to rapid development of 'flight or fight' response [80]. Exposure of rodents to natural predators or to their odors may induce stress-like states [1, 2]. Under such circumstances, there is rapid sympathetic activation leading to rise in the levels of adrenocorticoids in blood causing acute stress response to develop. Direct encounter with a predator has been effectively used to evaluate the biochemical and physiological changes produced during such stressful conditions [88]. Predatory stress in mice is induced by series of short exposures to natural predator like cat [16] or to any substance having the odor of cat like the fecal pellets of cat [14]. In one of the methods, mice are placed individually in different cages and after four initial 20-min cage habituation sessions each subject is submitted to two randomly assigned 20-min predator confrontation sessions. Change in behavioral pattern such as locomotion, shrieking-like voices. and endocrinological changes after the stress exposure are observed [16]. Another free-exploration test has been used, which consists of a PVC box (30 × 20 × 20 cm) covered with Plexiglas and subdivided into six equal square exploratory units, which are all interconnected by small entries [50]. It is divided half lengthwise by closing three temporary partitions. Approximately 20 h before cat exposure, each subject is placed in one half of the apparatus with the temporary partitions in place, in order to be familiarized with it. The floor of this half is covered with fresh sawdust and the animal is given unlimited access to food and water. On the test day, mice of each strain are randomly allocated to the following four groups:

(a) *Naïve + clay* animals are exposed to both familiar and novel compartments by removal of the temporary partitions. The novel compartment contains three odor-free clay pellets as models.
(b) *Naïve + feces* animals are exposed to both familiar and novel compartments. The novel compartment contains three cat feces pellets.
(c) *Exposed + clay* subjects are removed from the free-exploration box and confronted individually with a cat during a 5-min session. The cat cage consists of a PVC box (82 × 56 × 62 cm) subdivided into two compartments, one containing the cat and the other the mouse. Separation consists of a transparent PVC wall with holes allowing the cat to reach the other side with its paws. The mouse is then put back in the free-exploration apparatus and is exposed 1 h later to both familiar and novel compartments. The novel compartment contains three modeling odor-free clay pellets.
(d) *Exposed + feces* same as previous group, but the novel compartment contains three pellets of feces from the cat used during exposure. The behavior of the mouse is observed under red light for 5 min.

Marmosets (*Callithrix penicillata*) have also been employed for induction of predatory stress in a test battery known as Marmoset Predator Confrontation Test (MPCT) [23]. This model compares the behavioral response of experienced versus naive adult black tufted-ear marmosets in confrontation with a taxidermized wild-cat predator stimulus. After four initial 20-min cage habituation sessions, each subject is submitted to two randomly assigned 20-min predator confrontation sessions. Confrontation with the predator induces significant behavioral changes; i.e., proximic avoidance and tsik-tsik alarm call. Anti-stress drug administration, concomitant to predator exposure, reverses the behavioral changes observed [11]. Predator-induced stress is an established model to induce short-term acute stress response but its major disadvantage is development of habituation to predator exposure; hence, the use of this model for inducing stress is justified for developing only acute stress.

Day–night light change-induced stress (sleep deprivation-induced stress)

Changes in the circadian rhythm have profound effect on physical and psychological well being of an individual [8, 92]. Laboratory animals, when subjected to abrupt changes in day–night light pattern, exhibit acute stress response [43, 97]. Changes in circadian rhythms are regulated by pineal gland through the secretion of melatonin [13]. Melatonin is released from the pineal gland in response to dark or dim light whereas its functional antagonist serotonin is secreted in response to bright light. The serotonin–melatonin cycle is responsible for regulation of sleep–awake state of the body [12, 50]. Sleep deprivation is documented to be responsible for causing several forms of memory deficits [86, 105, 113]. There is an increase in the hypothalamic and thalamic oxidative stress level following sleep deprivation [25], which in turn is responsible for the cognitive impairment.

To induce stress, cages of rat or mice are kept under bright light from 1900 hours over night (in the dark phase) and cages are kept in dark room with no light from 1200 hours in the light phase for 180 min for 7–10 days [87]. This method is suitable for inducing short-term stress response. Another modification made in the method is by using multiple platform method. Groups of 4–6 animals are placed in the water tanks (41 cm × 34 cm × 16.5 cm), containing 12 platforms (3 cm in diameter) each, surrounded by water up to 1 cm beneath the surface, for 72 h. In this method, the animals are able to move inside the tank, jumping from one platform to the other [107]. Generation of stress can be evaluated by measuring the biochemical parameters associated with chronic stress response [104]. The major disadvantage of this model is that it can be effectively used only to generate short-term stress response, as on repeated exposure to this type of stressor the animal adapts to the changed day–night light pattern. This major drawback can be minimized by using this model as a part of chronic unpredictable stress protocol.

Noise-induced stress

Noise is one of the most widespread sources of environmental stress in living environments [131]. A large number of people are exposed to potentially hazardous levels of noise levels in daily modern life. Acute noise exposures activate the autonomic and hormonal systems, leading to temporary changes such as increased blood pressure, increased heart rate and vasoconstriction. After prolonged exposure, susceptible individuals in the general population may develop permanent effects, such as hypertension and ischaemic heart disease that are associated with exposures to high sound pressure levels.

Experimental studies have demonstrated ultra structural modifications in rat cardiomyocytes mainly in mitochondria due to noise stress. These sub cellular alterations are related to an imbalance in calcium homeostasis, which is supposed to be sustained by increased catecholamine innervations [100]. When noise exposure of any kind exceeds 90 dB, noise becomes a stressor. Noise stress has a depletory effect on free radical scavenging enzymes in the brain leading to moderate to severe oxidative stress [112] which can be a potential basis for hearing loss [35]. Studies have revealed that the exposure to noise stress alters the biogenic amine levels in discrete region of the brain [114].

Noise stress in laboratory rats can be produced by loudspeakers (15 W), driven by a white noise generator (0–26 kHz), installed 30 cm above the cage. Thus, a noise level can be set at 100 dB or above uniformly throughout the cage and can be monitored using a sound level meter. Each animal to be treated is exposed to noise stress for 4 h/day for 15 days. An acute model has also been developed involving exposure of rats to noise stressor of 10 kHz, 100 dB stress for 30 min [117, 118]. Control group rats are also kept in the above-described cage during the corresponding period of time, without noise stimulation to avoid the influence of handling stress on evaluation of effects due to noise exposure [85, 106]. The effect of noise stress exposure can be determined by estimating the brain biogenic amine level.

Post-traumatic stress disorder

Post-traumatic stress disorder (PTSD) is a debilitating anxiety disorder which develops in a subset of people after they experience emotional trauma. Several animal models of PTSD have been developed based on exposure to predator stress and social stress [41]. The studies have shown that inescapable exposure of cat (predator) to immobilized rats elicits intense fear in rats [16]. It is associated with inhibition of hippocampal functioning and enhancement of amygdaloid activity [1, 101], which is a clinical feature of PTSD [19]. A core symptom of PTSD is the repeated re-experiencing of the traumatic event, such that patients often feel as if the trauma is actually happening at the present. Therefore, rats are given a second inescapable cat exposure episode 10 days after the first to provide them with a reminder of their traumatic experience.

The rats are immobilized in Plexiglas enclosure (20 × 20 × 8 cm) and are taken to the cat housing room where they were placed in a metal cage (24 × 21 × 20 in.) with an adult cat for 1 h. The Plexiglas enclosure prevents any contact between the cat and rats, but the rats are exposed to all non-tactile sensory stimuli associated with the cat. The rats are exposed to same stressor after 10 days.

The predictability is an important factor in PTSD development and expression [44]. Therefore, to minimize the predictability of cat exposure, second exposure to cat is done during the dark cycle as compared with first exposure in light cycle. Unstable housing is an important factor in producing the full PTSD-like behavioral and physiological profile [29, 140]. Therefore, rats in stress are exposed to unstable housing conditions by randomly arranging in different cage–mate pairs on a daily basis and no rat in the stress group encounters the same cage-mate for two consecutive days [111]. The major limitation is that cat exposure in conjunction with immobilization lacks sense of horror and helplessness during trauma that is commonly reported by PTSD patients. Though these animal models present behavioral alterations resembling PTSD, yet, they fail to show the most consistent neuroendocrinologic characteristics observed in PTSD patients, namely, enhanced inhibition of the HPA axis.

Liberzon et al. [79] proposed single-prolonged stress (SPS) as an animal model of PTSD and rats exposed to SPS show enhanced inhibition of the HPA system. The typical SPS procedure for creating animal model of PTSD [132, 135] consists of a 2-h restraint in an acrylic animal holder, followed immediately by 20 min of forced swimming (temperature 25°C, water depth 40 cm). Thereafter, the rats are allowed to recuperate for 15 min. Then the rats were exposed to ether vapor until loss of consciousness and then placed in their cages and left undisturbed until the experimental manipulations [78].

Chronic variable/unpredictable stress models

The major disadvantage of both physical stress models and psychological stress models is the development of adaptation/resistance on chronic exposure. The changes in physiological and behavioral responses to chronic stress can be related to the adaptation of the HPA axis. When the same stressor is repeated, the HPA response undergoes desensitization or become stable as it has been reported that rodents repeatedly exposed to restraint stress exhibited a habituated corticosterone response, when they were subsequently challenged with an acute exposure to restraint [42, 82]. The other hand, the exposure to a multiple stress paradigm produces continued elevation in corticosterone levels, when the animals were subsequently subjected to acute restraint stress [4]. It has also been suggested that the adaptations of HPA axis depend on type, duration and severity of the stress regime [4, 17]. To prevent the development of resistance, chronic unpredictable stress models have been developed, which involve the use of various physical and psychological stressors in a predetermined manner so that the animal is not able to adapt to the stressor. Different research groups have employed different stressors for variable periods such as 10 days [87], 21 days [77] and 40 days [125].

In chronic unpredictable stress protocol for 10 days, animals are subjected to different stressors over a period of 10 days. One of the following stressors are administered daily (in random order) over a period of 10 days, like restraint stress, cold isolation, swim stress, sleep deprivation, food/water deprivation etc. more clearly from (Table 1). Similarly, in chronic unpredictable stress protocol for 21 days, animals are subjected to different stressors over a period of 21 days. One of the following stressors are administered daily (in random order) over a period of 3 weeks: fasting food deprivation for 20 h; water deprivation for 17 h; swimming at 4°C for 5 min; heat stress (40°C) for 5 min; 45° cage tilt for 17 h; shaker stress (horizontal shakes at high speed) for 10 min; restraint stress for 2 h; soiled bedding (200 mL water in 100 g sawdust bedding) for 5 h; persistent illumination (light for 17 h); tail pinch for 2 min; and intermittent white noise for 5 min. Immediately after each stress session, the rats are returned to the single room and maintained in standard conditions until the next session of the chronic unpredictable stress regime [77]. In another chronic unpredictable stress protocol for 40 days, animals are subjected to one stressor per day, at different times each day, in order to minimize predictability. The following stressors are used: (a) 24 h of water deprivation, (b) 1–3 h of restraint (c) 1.5–2 h of restraint at 4°C, (d) flashing light during 120–210 min, (e) isolation (2–3 days), (f) inclination of the home cages at a 45° angle for 4–6 h, (g) damp bedding (300 mL water spilled onto bedding during 1.5–2 h). Restraint is carried out by placing the animal in a

Table 1 The 10-day protocol employed in chronic unpredictable stress

Day	Stress type and schedule
1	1900 hours (previous night), humid sawdust, overnight; 1000 hours restraint, 60 min
2	1500 hours, cold (4°C) isolation, 60 min; 1900 hours, lights on, overnight
3	1200 hours, lights off, 180 min; 1500 hours, swim stress, 4 min
4	0730 hours, humid sawdust, all day; 1900 hours, food/water deprivation, overnight
5	1300 hours, swim stress, 3 min; 1900 hours, isolation housing, overnight
6	1400 hours, cold (4°C) isolation, 15 min; 1500 hours, lights off, 120 min
7	1900 hours, humid sawdust and lights off, overnight
8	1900 hours, isolation and food/water deprivation, overnight
9	1600 hours, restraint, 60 min; 1900 hours, lights on, overnight
10	0900 hours, swim stress, 4 min; 1000 hours, restraint, 60 min

Table 2 Representation of 40-day protocol for inducing chronic unpredictable stress

Day	Type of stress applied	Time of stress applied
1	Cold restraint	1.5 h
2	Inclination of home cages	4 h
3	Flashing light	2 h
4	Restraint	2 h
5	Isolation	Full day
6	Isolation	Full day
7	Isolation	Full day
8	Damp bedding	2 h
9	Inclination of home cages	6 h
10	No stressor applied	Full day
11	Flashing light	2 h
12	Water deprivation	24 h
13	Restraint	3 h
14	Damp bedding	3 h
15	Inclination of home cages	4 h
16	Cold restraint	2 h
17	Flashing light	3 h
18	Restraint	2.5 h
19	Damp bedding	3 h
20	Isolation	Full day
21	Isolation	Full day
22	Isolation	Full day
23	Cold restraint	1.5 h
24	Water deprivation	24 h
25	Inclination of home cages	4 h
26	Restraint	3 h
27	Flashing light	3 h
28	Restraint	1 h
29	Damp bedding	2 h
30	No stressor applied	Full day
31	Water deprivation	24 h
32	Inclination of home cages	6 h
33	Flashing light	2 h
34	Cold restraint	2 h
35	Isolation	Full day
36	Isolation	Full day
37	Isolation	Full day
38	Flashing light	3 h
39	Damp bedding	2 h
40	Restraint	3 h

25 × 9 × 7 cm plastic tube and adjusting it with plaster tape on the outside, so that the animal is unable to move. There is a 1-cm diameter hole at the far end for breathing. Exposure to flashing light is made by placing the animal in a 50-cm high, 40 × 9 × 60 cm open field made of brown plywood with a frontal glass wall. A 40-W lamp, flashing at a frequency of 60 flashes/min, is used [125] (Table 2). Some researchers have used exposure to predator odor-induced stress as a part of CUS protocol, in which mice are placed in a novel cage containing cat litter soiled with cat feces and urine [5]. Various authors have modified the stress models in order to accommodate them in their respective chronic unpredictable stress protocol. Other additional stressors that have been applied as a part of chronic unpredictable stress protocol are tail pinch with a clothes-pin placed 1 cm distal from the base of the tail for 5 min, strong illumination during predicted dark phase for 12 h, movement restriction in a small cage (11 cm × 16 cm × 7 cm) for 2 h, ether anesthesia until loss of reflex [74] and subcutaneous 0.9% saline injection [74].

Conclusion

None of the preclinical models is able to entirely reproduce different aspects of stress response as observed in clinical setup. Some models reproduce physical stress successfully and associated neuroendocrine changes, whereas others better reproduce the psychological stress and associated behavioral changes. Furthermore, animals tend to develop adaptation in response to same type of stressor. In these conditions, chronic unpredictable models are more advantageous because models involve the use of various physical and psychological stressors in a predetermined manner so that the animal is not able to adapt to the stressor. The psychological models offer advantage over physical methods with regard to ethical issue and pain inflicted during stress protocols. Nevertheless, the development of different animal models of stress has paved a way for identifying the effective therapeutic agents for ameliorating stress-related behavioral and pathological changes. Furthermore, the mechanisms involved in stress adaptation are not yet clear and these models are also helpful in delineating the mechanisms involved in stress adaptation also.

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