EXPERIMENTAL MODELS OF STRESS: OPPORTUNITIES, ADVANTAGES AND DISADVANTAGES (LITERATURE REVIEW)

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Stress is a state of mental or emotional strain resulting from adverse or demanding circumstances, triggered by various environmental, psychological, or physiological factors. Stress-related disorders represent a significant public health concern due to their negative impact on individuals, healthcare systems, and society. Understanding of the biological mechanisms, that form the basis of the stress and anxiety reactions, is essential for developing effective therapeutic interventions. Experimental animal models are indispensable tools for studying the neurobiological and behavioral consequences of stress exposure, allowing the replication of specific aspects of human stress responses under controlled laboratory conditions.

***The aim** – to summarize, classify, and compare the main experimental stress models used in animal research, highlighting their advantages, limitations, and translational relevance to human stress conditions.*

***Conclusion.** Animal models of stress, such as immobilization, cold exposure, forced swimming, foot shock, and neonatal isolation, are valuable for understanding the physiological and behavioral adaptations to acute and chronic stress. Each model reproduces distinct facets of human stress responses; however, none can fully replicate the complexity of human psychological stress. Therefore, combining different models and refining experimental protocols is essential for enhancing translational validity and improving research outcomes.*

***Key words**: stress; animal models, rodent, physiologic stress model, psychological stress model.*

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ЕКСПЕРИМЕНТАЛЬНІ МОДЕЛІ СТРЕСУ: МОЖЛИВОСТІ, ПЕРЕВАГИ І НЕДОЛІКИ (ОГЛЯД ЛІТЕРАТУРИ)

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Розлади, пов'язані зі стресом, становлять серйозну проблему громадського здоров'я, оскільки негативно впливають на окремих людей, систему охорони здоров'я та суспільство загалом. Розуміння біологічних механізмів, що лежать в основі стресових і тривожних реакцій, є важливою умовою для створення ефективних профілактичних і терапевтичних підходів. Експериментальні моделі є незамінним інструментом для вивчення нейробіологічних та поведінкових наслідків стресу у тварин, оскільки дають змогу відтворити окремі аспекти реакцій організму людини в контрольованих лабораторних умовах.

***Мета роботи** – узагальнення, класифікація та порівняння основних експериментальних моделей стресу, що застосовуються у дослідженнях на тваринах, з визначенням їх переваг, недоліків та відповідності умовам, наближеним до людського стресу.*

***Висновок.** Моделювання стресу на тваринах, зокрема, іммобілізація, вплив холоду, примусове плавання, електрошок та ізоляція новонароджених, є цінним засобом для дослідження фізіологічних та поведінкових адаптацій до гострого та хронічного стресу. Кожна з них відтворює певні аспекти стресових реакцій людини, однак жодна не здатна повністю охопити складність психологічного стресу. Тому поєднання різних моделей і вдосконалення експериментальних підходів є важливим для підвищення їх наукової достовірності та ефективності досліджень.*

***Ключові слова:** стрес, тваринні моделі, гризуни, модель фізіологічного стресу, модель психологічного стресу.*

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Introduction

Stress is a complex phenomenon that encompasses not only psychological but also physiological, endocrinological, and behavioral dimensions. Each of these dimensions requires specific measurement methods. For instance, psychological stress can be measured using questionnaires and psychometric tests, while physiological stress can be assessed through hormone concentrations, brain waves, or cardiac activity. The behavioral dimension, on the other hand, can be indirectly inferred through observations, such as changes in voice tone. Understanding the multidimensional nature of stress is crucial for developing effective measurement techniques. The lifetime prevalence of traumatic stress ranges from 0,56% to 6,67% in Europe, with high prevalence rates in the Netherlands, the UK, France, and Germany [1].

Stress – a term originally derived from mechanics, referring to the wear and tear on materials - can have serious consequences. These effects extend beyond the increasing incidence of modern diseases; it's also crucial to consider their impact on the brain. Persistent pressure and demands on individuals can lead to chronic stress, which floods the hippocampus - a vital brain structure involved in learning and memory - with cortisol, a stress hormone. This excessive cortisol can damage nerve endings and weaken the connections between nerve cells.

In the medium term, chronic stress can alter brain function, impairing concentration and making it difficult for affected individuals to recall information. Additionally, stress can worsen the conditions of patients with other diseases, such as multiple sclerosis, dementia, and various affective disorder [2, 3]. The main components of the stress system are the HPA axis and the locus coeruleus-norepinephrine/autonomic systems. Activation of the stress system leads to behavioral and physiological changes responsible for the adjustment of homeostasis [4]. Stressful life events may lead to the onset of severe psychopathologies in humans. Experimental models using animals, such as rats, reveal key molecular targets critical for identifying new therapeutic targets.

The aim of research

To summarize, classify, and compare the main experimental stress models used in animal research, highlighting their advantages, limitations, and translational relevance to human stress conditions.

The main part

Animal models of stress, which partially mimic the physiological and behavioral changes induced by stress in humans, have the potential to impact our understanding of human health significantly. These models, categorized as acute and chronic based on the duration of stress, provide valuable insights. In acute models, a stressor is applied once for a brief period, while chronic stress involves the repeated application of stressful stimuli over an extended duration [1, 5]. According to the organism's response, stress is divided into physiological, psychological, and comprehensive stress (Fig. 1). Restraint stress in animal models has been shown to produce inevitable physical and mental stress.

Cold stress. A series of abnormal changes in behavior, emotion, and the neuroendocrine system occur in response to cold stress. These findings are supported by studies such as [6], which found that cold stimulation affects productivity, oxidative resistance, and immune dysfunction [7- 9].

Practical and effective, the use of abrupt temperature reductions, whether through cold water or freezer compartments, has become a standard method to induce stress in laboratory animals. The most widely used protocols involve immersing the animals in cold water (15-18°C for 15-30 min) or placing them (in their home cages) in a cold, isolated environment (4°C for 15-30 min). This procedure can be applied in both acute and chronic protocols (7-14 days) [5, 10-12], demonstrating its versatility and practicality in research settings.

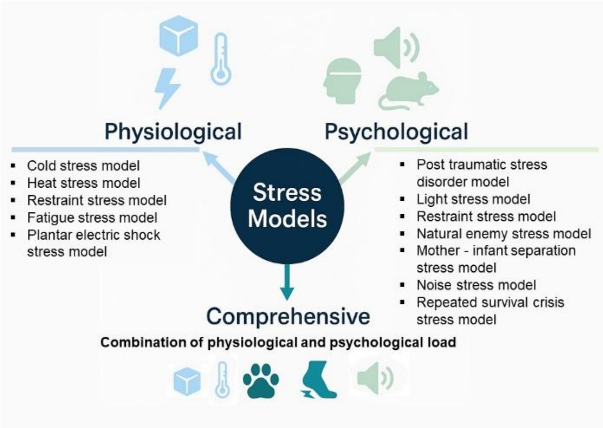

Fig. 1. Animal stress models (created by authors)

Heat Stress. Heat stress, resulting from elevated ambient temperatures (35-38°C), is a significant environmental stressor that triggers a severe systemic inflammatory response, underscoring the urgency of understanding its effects on homeostasis [13]. Research has shown that heat has multiple adverse effects on physiological function, immune function, the central nervous system, gut microbiota, and reproductive functions [14-16], highlighting the need for further investigation in this area.

Restraint Stress. In studies, using restraint stress, rats are placed in a small Plexiglas enclosure with access to air through their noses, causing them stress. It's important to note that these studies are conducted with strict adherence to ethical guidelines and regulations to ensure the welfare of the animals. To induce chronic stress over a period of 7-14 days, the animals' activity was reduced by placing them individually in a Ziploc bag and sealing the edges with tape. Acute stress was induced by employing the same method for a duration of 2 hours [17, 18].

Restraint stress has been found to lead to significant oxidative damage in central neurons, disruption of the endocrine system, and immune dysfunction, with potential implications for understanding stress-related diseases.

Immobilization stress. For acute stress induction, a single episode of immobilization typically extends over a significant period of 120-150 minutes.

The immobilization procedure involves securing the four limbs of the mouse/rat in the prone position on a plain board using adhesive tape. Additionally, a metal loop is fastened over the neck area to restrict head movement. This model, with its adjustable duration, is a widely used method for inducing both acute and chronic stress.

Rats and mice in laboratory settings demonstrate a high tolerance to immobilization stress, making them ideal candidates for chronic stress studies that span weeks or even months. This stressor, while sufficiently intense to activate the stress-responsive system in the body, including the HPA axis and the sympathetic nervous system, is well-tolerated by these animals, ensuring ethical considerations are met.

However, the limitation of the model is that the intensity of the stressor cannot be altered, unlike other stresses such as foot-shock stress or cold stress [19, 20].

Single Prolonged Stress. The single prolonged stress method involves subjecting animals to significant stressors in a controlled environment. The animals are placed in separate Ziploc bags with taped edges for a 2h period of immobilization. The animals are then required to swim in a cylindrical water tank for 20 min. Subsequently, a 15-minute recovery period is provided before the animals are released. The animals then undergo anesthesia and are left undisturbed for 7 days to allow researchers to observe the development of posttraumatic stress disorder symptoms without increasing mortality rates [17, 21].

Fatigue Stress Model. There are two different classifications of exercise-induced fatigue in the human body: physiological and psychological. These classifications are not just theoretical distinctions; they have practical implications in understanding and managing fatigue. They are distinguished by their triggering mechanisms and fatigue performance. A decline in motor ability characterizes physiological fatigue, whereas behavioral changes mark psychological fatigue. According to their etiology, exercise-induced fatigue is mainly divided into central fatigue and peripheral fatigue. They originate from two main pathways: one is through the central nervous system, and the other is through the peripheral nervous system, which involves muscles [22, 23].

The construction of central fatigue animal models is not just a theoretical exercise. It has practical applications in understanding and managing fatigue. This process primarily involves four methods: excessive exercise [24], neurotransmitter injection, immune inflammation [25], and sleep deprivation [26]. These methods, as demonstrated by Tanaka et al., are crucial in establishing an animal model of fatigue. For instance, Tanaka et al. kept rats in a cage filled with water to a height of 1,5 cm and selected a weight-loaded forced swimming test for evaluation of the extent of fatigue [27]. This practical application of the research makes the findings more relevant and useful to the audience.

The research process is meticulous and thorough, as demonstrated by Moriura et al. They employed a modified forced swimming test, where the rats swam with a load of steel rings that weighed approximately 8% of their body weight and were attached to their tails. The swimming time from the beginning of swimming with weight until the point at which rats could not return to the surface of the water, 10 seconds after sinking, was measured. Then, rats were helped out of the cylinder and returned to their home cage for recovery [28]. This level of detail and thoroughness in the research process ensures the reliability of the findings.

Electric foot shock as a physical stressor. Electric foot shock, when applied directly to animals, induces physical stress, resulting in behavioral and physiological changes. This stressor, unlike other commonly used stressors such as immobilization and restraint, offers a significant advantage in its predictability. The intensity, duration, and frequency of the shock can be varied, providing reassurance that stress of varying degrees can be induced.

Rodents are highly susceptible to mild shocks, exhibiting a remarkable response to stress following foot shock delivery. The protocol consists of placing rodents in a chamber with a metal grid floor connected to a shock generator.

Four electric shocks of 0,8 mA intensity and 2 s duration, delivered at an inter-stimulus interval of 1 min, have been shown to significantly trigger anhedonia-like behavior, a condition characterized by a reduced ability to experience pleasure, producing depression in rats [29]. Acute electric foot shocks with an intensity of 3 mA, duration of 200 ms, and a frequency of 1/s over 5 minutes to significantly increase the plasma corticosterone levels in rats [30].

One hundred twenty electric foot shocks, each of 0,15 mA intensity and 5 s duration, over a period of a 1-h session (every 30 s) were employed to investigate the effects of stress in altering the humoral and cellular immune function. The findings from this study by Brevet [31] are significant, as they provide insight into the immune system's response to stress, which is crucial for understanding the physiological effects of stress.

Neonatal Social Isolation Stress. The early-life stress of neonatal isolation in rats is a crucial area of research, as it produces immediate and long-lasting neural and behavioral effects. This research is significant in understanding the impact of early-life stress on neural and behavioral outcomes.

The brief isolation of an individual pup from the dam and litter, when repeated for several days, has been proven to be an effective method of stimulating the neonatal HPA axis. Importantly, this method does not alter weight or growth in either neonates or adults [32, 33]. The reliability of the method instills confidence in the research outcomes.

During the neonatal separation procedure, on the 2-nd day after birth, the litter of the inbred strain is removed from the cage and placed in another cage for 1 hour (9 a.m./12 p.m.) in a room located apart from the animal facility. After 1 hour, the litter is returned to their dams in their home cages. The separation procedure is repeated for 8 days. This model has been used extensively to demonstrate the effect of early lifetime stress on vulnerability to addiction, a condition where an individual is more likely to develop a substance use disorder, and in the generation of anxiety-like behaviors [11, 34].

Conclusions

Despite their limitations, animal models are indispensable in advancing our understanding of stress-related diseases, as underscored in this review. The nature of the experiments influences the choice of animal model, the research methods employed, research expertise, and awareness of the ethical considerations associated with using animals as models. An ideal animal model for stress should accurately predict all aspects of the stress response and naturally replicate disease development.

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