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| RESEARCH ARTICLE

Inflammation, Oxidative Stress, and Chronic Unpredictable Mild Stress Model of Depression

Vlad Dionisie
Assistant Lecturer, Department of Psychiatry and Psychology, "Carol Davila" University of Medicine and Pharmacy, 020021 Bucharest, Romania
Corresponding Author: Vlad Dionisie, **E-mail**: vlad.dionisie@gmail.com

| ABSTRACT
Depression is one of the most common psychiatric disorders. Animal models represent a valuable research tool to investigate the molecular pathogenic mechanisms of depression and to develop and test different possible antidepressant drugs. Therefore, several murine models have been established, but the chronic unpredictable mild stress model has robust evidence. Inflammation and oxidative stress pathways have a clear involvement in the complex and intricate pathophysiology of depression, with strong evidence coming from studies using the chronic unpredictable mild stress model. This review provides an overview of the characteristics of the chronic, unpredictable mild stress model of depression and its role in investigating inflammation and oxidative stress pathways in depression.

| KEYWORDS
Depression, Oxidative stress, Inflammation, Chronic stress, Animal model.

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1. Introduction

According to World Health Organization estimates, unipolar depressive disorders are the leading cause of disability globally and will also become the main source of burden of illness by 2030 (Lépine & Briley, 2011; WHO, 2017). Depressive disorders led to more than 50 million years of living with disability globally in 2015 (WHO, 2017). Depression affects 322 million people globally, resulting in a prevalence of 4.4%. Prevalence variations by region are quite small, with estimates ranging from 5.4% (in the Africa region) to 3.6% (in the Western Pacific region). The lifetime risk of developing a major depressive episode is 15-18%, i.e. 1 in 5 people will experience a depressive episode during their lifetime (WHO, 2017).

At present, a full understanding of the pathophysiological mechanisms of depression is not possible, so creating an animal model that fully mimics the disease is impossible. This is also difficult because mental disorders are partly defined by subjective experiences. Experimental models of depression now in use attempt, by various means, to reproduce symptoms from human to animal in a quantifiable way. Current animal models for depression vary considerably in the degree to which they succeed in producing depression-like features. Parameters that can be quantified in mouse or rat behavioral models are the behavioral (motor) response to stress, the reward/pleasure response and social interaction. These parameters are thought to reflect the degree of helplessness or hopelessness, anhedonia and social withdrawal observed in patients with depression (Vlad Dionisie, 2022).

Animal models of depression are evaluated by three major criteria: etiological/construct validity (how well the model replicates the underlying mechanisms of the disease in humans), predictive validity (the animal's response to a given drug can predict the response to administration in humans), symptomatic/face validity (the degree of symptomatic similarity between the model and the patient, the ability to reproduce in animals the clinical aspects in humans). Revision of these "classic criteria" proposed

additional criteria: homological validity, pathogenic validity, mechanistic validity, convergent validity, discriminant validity, internal and external validity (Becker et al., 2021; Willner, 1984; Willner & Mitchell, 2002).

The degree of etiological validity of current animal models of depression is quite low, mainly because the pathogenic mechanisms of depression are very complex, intricate, and incompletely understood. Most often, animal models of depression attempt to replicate one or a few of the etiological pathways of the disease. At the same time, some animal models of depression can achieve a higher degree of symptomatic validity, but the way in which this is achieved is not similar to the conditions under which depression occurs in humans (Vlad Dionisie, 2022).

Current literature describes four classes of animal models of depression: 1. Acute or subchronic application of stressors (despair-based models - forced swimming, tail suspension); 2. Chronic application of stressors (chronic, environmental and unpredictable stress, chronic social isolation, etc.); 3. Administration of chemical compounds with known depressogenic effects (lipopolysaccharide, reserpine, cytokines, etc.); 4. Genetic or surgical modelling (olfactory bulbectomy, genetic line of stress-sensitive Flinders rats, etc.) (Vlad Dionisie, 2022).

Exposure to stress is the main environmental risk factor associated with the development of depression in humans (Bentley et al., 2014; Meng et al., 2017). For this reason, many researchers have attempted to create animal models of depression using psychological stress exposure. These models are considered to have a much higher degree of analogy with the clinical situation.

2. Chronic unpredictable mild stress murine model of depression

The paradigm of medium and unpredictable chronic stress was developed to study neurobiological changes caused by psychological stress applied over a longer period. This animal model of depression was developed about three decades ago by Katz R. (Katz, 1982). This model was designed to induce the anhedonia-like behavior and loss of interest in reward observed in depressed patients. Along with these changes, animals also exhibit other manifestations that may be associated with the patients' clinical symptoms: changes in physical activity and decreased fur condition or sexual activity. Behavioral manifestations are induced by the application of various stressors over a period of 3-9 weeks and in an unpredictable and uncontrollable manner for the animal. This avoids the emergence of coping mechanisms that usually develop following the continuous application of a single stimulus. At the same time, the stimuli applied are of medium intensity, similar to those encountered by patients in everyday life. Compared to other models based on chronic application of stressors, this model has the highest translational capacity (Frisbee et al., 2015).

The natural preference of mice or rats for sweet solutions is thought to be the equivalent of human pleasure and reward behavior. Thus, a decrease in the total amount of sweet solution consumed after exposure to a chronic stress protocol is considered a sign of depression (Grønli et al., 2004). Other signs of depressive symptoms may be decreased frequency of grooming (fur washing, coat hygiene status, etc.), reduced sexual activity, aggression, motor inhibition, decreased exploratory/investigative behavior (Mutlu et al., 2012).

The way this model has been constructed offers the possibility of combining many stressors over different periods of time. Stressors that can be applied include water and/or food deprivation, cage tilt, intermittent lighting, wetting of cage bedding, housing more than one animal in the same cage, forced swimming, etc. (Yan et al., 2010). Chronic but not acute administration of antidepressants brings sucrose consumption and other behavioral signs to normal, which best mirrors the clinical course of patients on antidepressant treatment (Yan et al., 2010).

Thus, this model has good predictive (animals respond to antidepressant treatments), symptomatic (replicates almost all symptoms that can be replicated), and etiological validity (replicates etiological mechanisms observed in humans, such as corticosteroid resistance, neuroinflammation, oxidative stress, neuroplasticity). Also, the meta-analysis by Antoniuk et al. showed that the chronic, environmental, and unpredictable stress paradigm is a robust animal model of depression and is strongly associated with anhedonia behavior in rats (Antoniuk et al., 2019).

However, the chronic medium and unpredictable stress animal model of depression also has drawbacks. On the one hand, it requires a lot of time and space in the laboratory to implement, and on the other hand, the reproducibility of the protocol and results can be difficult to achieve (Yan et al., 2010).

3. Oxidative stress (OS) and inflammation as pathogenic mechanisms in chronic unpredictable mild stress murine model of depression

OS is defined as the imbalance between reactive oxygen species (ROS) production and the antioxidant capacity of the cell. The most important source of ROS is the mitochondrial oxidative phosphorylation process that occurs in ATP synthesis processes. ROS

are free radicals or free anions/molecules containing oxygen atoms and are highly reactive towards many biological substrates (Lindqvist et al., 2017; Maes et al., 2011). ROS play an important role in intracellular signaling systems. Increased levels of ROS cause the inactivation of some enzymes, the peroxidation of lipids, and structural alterations of DNA. Thus, ROS can lead to structural and functional deficiencies of the cell and ultimately to apoptosis (Fridovich, 1986; Halliwell & Gutteridge, 2015). The brain is more vulnerable to SO because it has a high content of polyunsaturated fatty acids, a high energy requirement and a low reserve of antioxidants (Lee et al., 2020). It is believed that overproduction of ROS causes activation of pathological cascade mechanisms that will lead to increased blood-brain barrier permeability, brain morphological changes and neuroinflammation (Salim, 2017).

Chronic psychological stress produces hypothalamic-pituitary-adrenal (HPA) axis dysregulation. The link between stress, HPA axis dysregulation and inflammation is modulated by changes in SO. Specifically, the mechanism responsible for the occurrence of HPA axis dysregulation is reduced glucocorticoid receptor sensitivity. In the presence of hydrogen peroxide, nuclear translocation of the glucocorticoid receptor after dexamethasone administration was impaired. These data suggest the involvement of SO in the deregulation of the negative feedback loop of the HPA axis (Okamoto et al., 1999). At the same time, the appearance of pro-inflammatory cytokines is modulated by ROS in a nuclear factor kappa-light-chain- enhancer of activated B cells (NF-κB) pathway-dependent manner (Sanlioglu et al., 2001). On the other hand, catecholamine excess induced by psychosocial stress activates the synthesis of pro-inflammatory cytokines via increased NF-κB pathway activity. Although physiologically activation of the HPA axis leads to inhibition of the inflammatory response, the emergence of glucocorticoid resistance leads to hyperactivation of the HPA axis and perpetuation of neuroinflammation in affective disorders (Mucci et al., 2020).

The etiological validity of the experimental rat model of depression induced by chronic, environmental, and unpredictable stress in terms of oxidative and inflammatory mechanisms has been tested in numerous studies. Numerous authors have demonstrated that animals subjected to the chronic, medium, and unpredictable stress paradigm showed higher levels of inflammation in brain regions of interest for the study of depression. Specifically, higher levels of tumoral necrosis factor (TNF)-alpha, interleukin (IL)-6, IL-1beta, NOD-like receptor protein 3 (NLRP3) or NF-κB in the hippocampus or frontal cortex were observed in rats with depression induced by this type of protocol compared to control animals (Dai et al., 2020; Fernandes & Gupta, 2019; Li et al., 2017; Y.-M. Liu et al., 2017; Q. Wang et al., 2018; Xue et al., 2015). Regarding oxidative stress, several studies have shown that the chronic, mild and unpredictable stress animal model of depression showed changes in this direction, along with alterations in the antioxidant system. Most studies in the current literature showed that this animal model of depression is associated with an increase in oxidative stress parameters (e.g. malondialdehyde, protein carbonyl, thiobarbituric acid reactive substances) or a decrease in molecules involved in the antioxidant defense (total antioxidant capacity Trolox, glutathione, glutathione peroxidase, catalase, peroxiredoxins, superoxide dismutase) in the cerebral cortex, hippocampus or frontal cortex (Arent et al., 2012; Che et al., 2015; Fontella et al., 2005; Gill et al., 2018; J. Liu et al., 1996; Martín-Hernández et al., 2016; Matchkov et al., 2015; Rai et al., 2019; Scotton et al., 2020; C. Wang et al., 2012). Thus, these results mirror the changes detected clinically in human subjects and also give this model of experimental depression the necessary evidence to be used for exploring the oxidative-inflammatory mechanisms in depression.

5. Conclusion

This review outlined the main characteristics of the chronic unpredictable mild stress murine model of depression and its role in exploring inflammatory and OS pathways of depression. The chronic, unpredictable mild stress model is induced by environmental means (chronic stress) and represents a useful tool to investigate anhedonia. Moreover, several studies showed increased levels of inflammatory and oxidative stress markers in different brain regions of rodents with chronic stress induced depression. Future studies should aim at developing depression models that cover as many facets of human depression as possible, from symptoms to pathophysiology and treatment response.

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