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Review article

Between stimuli and responses: behavioral models of anxiety in animals. A descriptive review

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ABSTRACT

Entre estímulos y respuestas: modelos conductuales de ansiedad en animales. Una revisión descriptiva.

La ansiedad, como emoción adaptativa, puede motivar a individuos a buscar soluciones y mejorar su rendimiento, pero cuando alcanza niveles clínicamente significativos, afecta significativamente la vida diaria. El presente artículo de revisión descriptiva narra la importancia del estudio de la ansiedad a través de modelos animales a nivel preclínico, destacando su relevancia en la comprensión de la neurobiología y el desarrollo de tratamientos eficaces. Se realizó una búsqueda de información científica actualizada en bases de datos relevantes para el área de la salud, de los últimos años, como PubMed, Web of Science, Embase, SciELO, Cochrane Library, Google Scholar, entre otras. En él se discuten modelos experimentales de respuesta condicionada y no condicionada, utilizados para replicar características de estos trastornos en animales de laboratorio, los cuales deben poseer validez aparente, de constructo, y predictiva. Se destaca la limitación de estos modelos para replicar la experiencia humana completa de ansiedad, así como las diferencias fisiológicas y de comportamiento entre especies. A pesar de sus limitaciones, los modelos animales siguen siendo herramientas valiosas para comprender y tratar la ansiedad, aunque se requiere más investigación para mejorar su validez y aplicabilidad clínica.

ABSTRACT

Anxiety, as an adaptive emotion, can motivate individuals to seek solutions and improve their performance, but when it reaches clinically significant levels, it significantly affects daily life. This descriptive review article narrates the importance of studying anxiety through

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animal models at the preclinical level, highlighting its relevance in understanding the neurobiology and development of effective treatments. A search for updated scientific information was conducted in relevant health-related databases from the last years, including PubMed, Web of Science, Embase, SciELO, Cochrane Library, and Google Scholar, among others. It discusses experimental models of conditioned and unconditioned responses used to replicate characteristics of these disorders in laboratory animals, which must possess face, construct, and predictive validity. It emphasized the limitation of these models in fully replicating the complete human experience of anxiety and the physiological and behavioral differences between species. Despite their limitations, animal models remain valuable tools for understanding and treating anxiety, although more research is needed to improve their validity and clinical applicability.

INTRODUCTION

As an emotion, anxiety is an adaptive response to a potential danger in the environment, whose primary function is to prepare the organism through a state of alertness that leads to behavioral and physiological changes that allow the subject to seek appropriate survival strategies according to the perceived threat (1).

Moderate anxiety can motivate individuals to seek solutions to problems and enhance physical and intellectual performance, facilitating adaptation to new or unpleasant situations (2). However, when anxiety reaches a pathological level, it is interpreted as an excessive and inappropriate emotional response, wherein the perception of threat is amplified or misjudged, leading to disproportionate reactions (3). This pathological anxiety occurs unexpectedly and recurrently, even in the absence of threatening stimuli or when they do not represent any real danger, hindering the ability to handle difficult situations (4). Consequently, individuals experience significant difficulties in daily life, impacting functioning in various areas such as social, occupational, and family life. It is recognized as a common psychiatric disorder requiring clinical attention due to its characteristic symptoms, which lead to imbalances and dysfunctions at the physical, cognitive, and behavioral levels (5). Within the pathological anxiety previously described are anxiety disorders, which represent the most common mental disorders, involving a failure to choose an adaptive response and inhibit a maladaptive response to many diverse situations (6). The American Psychiatric Association (APA) (DSM-5) classifies anxiety disorders into separation anxiety disorder, selective mutism, specific phobia, social anxiety disorder (social phobia), panic disorder, agoraphobia, generalized anxiety disorder, substance/medication-induced anxiety disorder; anxiety disorder due to another medical condition; other specified anxiety disorder; and other unspecified anxiety disorder (7). Various neurotransmitters, such as GABA, glutamate, serotonin, and dopamine, play a crucial role in anxiety. Brain regions such as the amygdala, hippocampus, prefrontal cortex, and hypothalamus are also involved. Recent studies, including brain imaging, genetic studies, and animal models, have advanced our understanding of the neurobiology of anxiety, driving the development of more efficient treatments (8).

This descriptive review aims to provide an overview of the most animal models in the study of anxiety at the preclinical level, focusing on their design, validation and applications in preclinical research. In addition, the implications of these models for understanding human anxiety will be discussed, as well as their limitations and possible future directions.

METHODS

Eligibility criteria

The eligibility criteria were established by the corresponding author. A search was conducted for animal models assessing anxiety-like behavior. These models were assigned to the co-authors for the article selection process, and articles that provided detailed descriptions of the methodologies for each model were included. During the selection process, all members independently searched

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databases using keywords, reviewing the titles and abstracts of the retrieved references to identify relevant studies. The inclusion criteria focused on original preclinical studies evaluating anxiety-like models, with no restriction on publication years (we gave priority to studies from the past five years). Non-animal studies were excluded. The selected studies were validated by all authors, and in case of disagreement, a working meeting was convened to reach a consensus.

We researched the electronic databases PubMed, Web of Science, Embase, SciELO, Cochrane Library, Google Scholar, Dialnet, and Scholarpedia up to March 2024. The results were organized using citation management software (EndNote, Clarivate Analytics), where duplicate entries were grouped and eliminated. We prioritized articles published within the last 5 years; however, citing older articles, up to 10% of the total, was allowed due to their historical relevance or if they were considered essential for methodology. We identified the data from articles that we deemed relevant and synthesized them following an editorial order. We discarded data that we did not consider relevant.

Risk of bias

We reviewed the Cochrane risk of bias tool adapted for animal studies, assessing general quality aspects related to animal selection, blinding, data loss, and outcomes. However, the most important factor for this review was the detailed description of the methodologies.

RESULTS

Experimental models of emotional disorders

Experimental models of emotional disorders aim to replicate specific characteristics of human psychiatric disorders in laboratory animals by correlating physiological and behavioral changes. Due to evident cognitive differences between species, these models do not aim to fully replicate all aspects of a particular animal disorder (9). However, experimental models for anxiety are designed to replicate key features of human anxiety disorders to study their neurobiology and develop potential treatments. These models precisely measure physiological and behavioral variables that occur when animals are exposed to controlled laboratory conditions simulating natural and potentially anxiety-inducing situations. To achieve this, a series of instruments or tools, referred to as 'tests,' are used to measure anxiety-related parameters. Using these tests in a battery is recommended to evaluate the behavioral phenotype of animals under different conditions (10).

Rodent models of psychiatric diseases must possess face, construct, and predictive validity. This means that the measured behavioral variables should closely resemble the behavioral responses observed in humans (face validity). Furthermore, the model should exhibit sensitivity to pharmacological agents and elicit comparable effects (such as anxiolytic, anxiogenic, or no effect) in animal and human models (construct validity). Finally, the model should demonstrate a discernible response to drugs that are generally effective in humans, indicating similarities in the biological factors underlying the disorder between animals and humans (predictive validity) (11, 12).

Most proposed animal models of anxiety primarily adopt a behavioral approach and are classified based on conditioned or unconditioned responses to stimuli believed to induce anxiety in humans (4).

Animal models of anxiety

The number of studies on animal models of normal anxiety has increased in the decade from 2010 to 2020, demonstrating the relevance of these models. As mentioned above, animal models aim to replicate various characteristics of the human condition, including behavioral and physiological changes, which collectively constitute the emotional state (13, 14). Research on anxiety in animals is crucial because our understanding of its pathogenesis remains incomplete. Detailed neurobiological models enable the experimental manipulation of specific components in nerve transmissions underlying psychopathology and testing new drugs or compounds with clinical potential (13). A wide variety of animal models of normal anxiety

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are classified based on unconditioned responses and conditioned responses (11). The following summarizes the most widely used models for studying anxiety.

Models based on unconditioned responses

Ethological models aim to replicate the natural conditions under which emotional states of fear and anxiety arise. These models do not necessitate complex training, utilize non-painful aversive stimuli to induce anxiety, and leverage the animal's exploratory behavior in novel environments, measuring its instinctive responses to environmental challenges rather than its fear of potential consequences. The stimuli used to induce anxiety are diverse and may include exposure to brightly lit areas, heights, open spaces, or the scent of a predator, among others (15). The models are outlined below:

Models based on exploration

Elevated plus maze (EPM)

This structure is elevated off the ground as a plus sign, with two open arms (without walls) facing each other, and two closed arms (with high walls) facing each other. The animal encounters open and elevated spaces that create a conflict between its natural aversion to open areas and its drive to explore. This conflict results in hesitation and increased anxiety, as rodents are inherently wary of open spaces. The animal will avoid open arms. This tendency is suppressed with anxiolytics and enhanced with anxiogenic agents. The number of entries into the closed arm and motor activity are measured (13).

Elevated zero maze (EZM)

It is a modification of the elevated cross maze designed for mouse research. It is an elevated ring platform with two opposing open and two closed quadrants. Animals are placed in one of the closed quadrants (this eliminates errors or ambiguity in the interpretation of time spent in the center of the traditional design structure allowing for uninterrupted exploration). Behaviors are recorded both by the observer and through videotaping (16).

Improve elevated beam walking (IEBW)

This exploratory test examines high-level fear stress using an IEBW apparatus to examine the anxiolytic effect by installing a very narrow timber 190 cm above the floor. The apparatus includes open and closed arms. Rats are placed at the tip of the open arm 140 cm from the closed arm and their behavior is examined for 3 min. The most important variables are time spent in the open arm, low and high frequency (17).

Open field – hole board (OF-HB)

It originated as an exploratory test in the 1960s and was adapted later for neuropharmacological studies. The test consists of a base with holes evenly distributed within a high-walled box. The exploration is analyzed more specifically in which the "head dipping" variable is the most representative of an anxious behavioral response since the increase of this variable indicates an anxiolytic effect. In contrast an anxiogenic effect will be a decrease of the same. It separates anxiety, exploration, activity. and learning responses (14, 18).

Light/dark box test (L/D)

This test provides a conflict situation based on exploration and the rodent's natural behavior (active at night and avoids light). Although rats have a strong preference for darkness, mice are preferred in the light/dark box test due to their more consistent behavioral responses and sensitivity to light under experimental conditions. In this test, the rodent may avoid passing through a highly illuminated area or venture through it, leaving the dark compartment behind, depending on the balance between curiosity and anxiety. This test is susceptible to external factors, and some inconsistencies have been found. Light intensity and test duration can influence scanning, potentially compromising the detection of anxiolytic or anxiogenic effects. It measures transitions between light and dark versus time (19).

Actophotometer test (APhT)

Digital Actophotometer is a 5-minute test of a cube equipped with 12 infrared-sensitive photocells in two rows (in a room with no light or sound).

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Animals are placed inside the cube and locomotor activity readings are calculated regarding total photobeam counts (20).

Free exploration test (FET)

It is a box subdivided into six equal exploratory units interconnected by small doors; this can be divided in half longitudinally by closing three temporary partitions. The box on the floor is kept in an empty, noise-free room. The animals are placed in one half of the apparatus, and once habituated, the temporary partition is removed at 10 min. The behavior is recorded with a video camera under red light. The variables are the number of rearings, locomotion, stay in the novel area and the number of transitions from the known to the unknown environment (21). Some researchers have questioned the validity of FET as an exclusive measure of anxiety. It is argued that the behavioral changes observed in FET may be influenced by other factors, such as familiarity with the environment, the animal's exploratory motivation, or even individual characteristics such as breed or strain (21).

Mirror Chamber test (MCT)

The mirror chamber is a chamber (cube) usually made of wood with a mirror inside. A 5 cm corridor is formed in the center of the chamber. The animals are placed in a corner for 5 min, and the variables of latency to enter the mirror chamber, number of chamber entries, and total time in the mirror chamber are measured (22).

Models based on social behavior

Social interaction test (SIT)

The social interaction behavior of the mice is evaluated as follows: A mouse is placed for 10 min in a clear acrylic test box to habituate to the conditions. Then, an unknown mouse with the same weight is placed in a plastic box with holes (to allow social interaction) and a triangular prism shape between them in low light conditions. The variables of social sniffing, genital and anus sniffing, aggressive behavior, and social grooming are measured for 10 minutes (23).

Stress-induced modifications of behavioral and / or physiological responses models

Suppressed feeding test (SFT)

It is a conflict paradigm test based on hyponeophagia. After a 24-h fasting period, mice are placed in a novel cage (with the same type of wood chips on the floor) and in the center with a single food pellet. The mouse is in a corner, and their interaction latency with the pellet is recorded. A reduced latency to interact with the pellet is an anxiolytic effect (24).

Neonatal maternal separation model

It is significant in studying adverse early-life experiences, identified as risk factors for later-life mental illness. This model entails separating pups from their mothers during specific periods of the critical postnatal period, either for 3 hours per day or for an extended period of 11 hours on postnatal day 24. Neonatal rodents subjected to this maternal separation exhibit heightened anxiety-like behaviors compared to a control group (25, 26).

Others

Marble-burying test (MBT)

The MBT test measures anxiety about environmental challenges. Four glass marbles are placed along one side wall of each cage, and the behavior of the rats is observed for 30 min. The variables to be measured are the number of rats that bury marbles and the number of marbles buried. This behavior reflects an effort by the murine to hide the unknown object in the sawdust and thus may indicate anxiety-like behavior (27).

Models based on conditioned responses

The Pavlovian experiment serves as a classic illustration of conditioned responses. Pavlov demonstrated that a neutral stimulus can acquire affective properties through association with a biologically relevant stimulus. The acquisition of conditioned fear occurs when a neutral stimulus (such as light or sound) is paired with an aversive stimulus (typically a mild electric shock), resulting in both physiological changes (such as alterations in heart

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rate) and behavioral responses (such as freezing).. Repeated pairings make the neutral stimulus a conditioned stimulus that triggers conditioned responses. These responses often manifest as abnormal behaviors characteristic of anxiety disorders, such as hyperactivity, social withdrawal, and learning deficits, utilized to investigate the neurobiological mechanisms underlying fear and anxiety modulation in the brain (28). The models are as follows:

Fear potentiated startle reflex

Foot shock (FS)

The device is a transparent plexiglass chamber with a stainless steel grid floor connected to the standing shock delivery system. The rats were placed in the chamber for seven days and received a noise for 30 seconds, followed by five electric shocks to the feet (2 mA, 2 s) for 15 minutes. On days 7, 14, and 21, the animals are placed back in the same chambers and monitored with a video camera. The variables to be measured are the time dedicated to active movement and freezing (29).

Conditioned fear stress test (CFST)

A wooden box divided into three compartments with a stainless-steel mesh floor is used. Intermittent and inescapable electric shocks are applied to the grid. The variables to be measured are the duration of freezing behavior and motor activity by monitoring. This process is done 2 days prior to the actual test (30).

Maximal electroshock test (MET)

One hour after treatments, mice undergo transcorneal electrical stimulation (50 mA, 0.2 s: 60 Hz), which causes seizures. The variables to be measured are the flexion time of the forelimbs, the extension time of the hind limbs, incidence and lethality (31).

Rotarod test (RRT)

The rotarod is a rotating cylinder approximately 4.5 cm in diameter with a speed controller. The mice are placed on top of the cylinder and the rotarod is accelerated from 5 to 20 rpm with speed maintained for 5 min, and then subjected to higher speeds. The measurement variable is the time spent in the cylinder without falling (32).

Predator stress model

In the experimental setup, rats are exposed to visual, olfactory, and auditory cues associated with predators, while preventing physical contact or attack. Observations reveal anxiety-like behaviors in the rats, such as darting movements, cowering, stiffness, and a notable increase in excretion, all attributed to predator-related stimuli. Both acute and chronic exposure to predator stress result in heightened expression of corticotrophin-releasing hormone (CRH) mRNA, and others, indicating activation of the hypothalamus-pituitary-adrenal axis and the consequent manifestation of anxiety behaviors (25, 33).

Drug models

Altering the administration of drugs leads to changes in hormone and neurotransmitter levels in animals, disrupting their normal mechanisms and triggering anxiety. Prominent models for inducing anxiety include those caused by caffeine, lipopolysaccharide (LPS), and 5-HT (22, 34).

Limitations of the models

Behavioral tests to study anxiety-like responses in animals are valuable tools for understanding the mechanisms underlying anxiety disorders and for developing new treatments. However, these tests have some limitations that we must consider. One of them is the difficulty in replicating the complexity and subjectivity of the human experience: Anxiety in humans is complex, encompassing emotions, thoughts, and physical responses (4). Preclinical models often capture only one or two aspects of anxiety, which limits their ability to fully reflect the real experience. Moreover, differences in cognitive capacity and complexity across species further constrain their applicability. Some tests (such as models based on conditioned responses) may lack specificity because they detect not only anxiety but also other disorders such as depression or schizophrenia (4, 8). Direct handling by technicians

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could, in itself, cause anxiety, despite methodologies that involve familiarizing the animals with the presence and scent of the technician before testing. Another point to consider is that the physiology, behavior, and neurobiology of animals are different from those of humans. This means that results from preclinical models can only sometimes be directly applied to the clinic. Treatments effective in animals are not always effective in humans, and vice versa (8). As a final consideration, in addition to the limitations mentioned, researchers may need to work on selecting tests or models that are not optimal for their objectives, or due to cost constraints, they may not conduct multiple behavioral tests that could be complementary. Moreover, despite the disparity in the incidence of mental health problems between sexes, the trend towards reducing the number of animals could discourage the inclusion of both males and females. Efforts exist to reduce the ambiguity of individual tests, such as a recent unified scoring system for specific behavioral traits that maximizes the use of generated data while minimizing statistical error across different tests. Therefore, it is advisable to conduct a battery of behavioral tests rather than individual tests (35).

Prospects

We consider the study of anxiety in preclinical models to remain highly relevant for biomedical and neuropharmacological sciences, among others. These models serve as reliable methods to assess the efficacy of new pharmacological treatments and understand the brain mechanisms involved in their functioning. There is a surge in the investigation of pharmacological new drugs or combinations, which must be tested in these models before human trials. However, it is crucial to weigh whether the potential benefits of preclinical research justify the risk to experimental animals, particularly in repetitive studies with similar natural compounds whose effects have already been described. Researchers, ethics committees, and institutions funding the research must carefully consider this ethical balance. Finally, we believe it is necessary to develop preclinical models that more accurately replicate human experiences, including comorbidities such as depression, chronic pain, and substance abuse.

CONCLUSIONS

Animal models have many positive characteristics, including being easy to obtain, maintain and handle, relatively inexpensive, and exhibiting greater reproducibility than clinical studies. There is ample evidence of similarities between rodents and humans in terms of the neural circuits and hormonal responses involved in stress and anxiety, particularly the functioning of the hypothalamic-pituitary-adrenal (HPA) axis and key brain regions such as the amygdala and prefrontal cortex. Additionally, aspects related to equivalences in terms of basic cognitive processes and anxiety-related behaviors such as conditional learning, anticipation of danger, and avoidance are highlighted. Freezing and escape are essential survival mechanisms. However, some limitations must be considered. It is recommended to interpret the results with caution, considering that they are not always directly applicable to human anxiety. Further research is necessary to develop more valid and specific preclinical models and improve the translation of results into clinical practice.

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