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REVIEW

# Recent advances in anxiety disorders: Focus on animal models and pathological mechanisms

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## Abstract

Anxiety disorders have become one of the most severe psychiatric disorders, and the incidence is increasing every year. They impose an extraordinary personal and socioeconomic burden. Anxiety disorders are influenced by multiple complex and interacting genetic, psychological, social, and environmental factors, which contribute to disruption or imbalance in homeostasis and eventually cause pathologic anxiety. The selection of a suitable animal model is important for the exploration of disease etiology and pathophysiology, and the development of new drugs. Therefore, a more comprehensive understanding of the advantages and limitations of existing animal models of anxiety disorders is helpful to further study the underlying pathological mechanisms of the disease. This review summarizes animal models and the pathogenesis of anxiety disorders, and discusses the current research status to provide insights for further study of anxiety disorders.

**KEYWORDS**
animal models, anxiety disorders, behavioral tests, mental diseases, pathogenesis

## 1 | INTRODUCTION

Anxiety disorders are one of the major psychiatric disorders characterized by persistent psychogenic anxiety, somatic anxiety, and sleep disturbance. Estimates of current prevalence range between 7.3% and 28.0%, and the World Health Organization has ranked anxiety disorders as the sixth-largest contributor to global disability.[1] Meanwhile, the COVID-19 pandemic has further exacerbated the burden of global psychiatric disorders. The prevalence of anxiety disorders increased by ~76 million in 2020, an increase of about 25.6%.[2] Prevention and treatment of anxiety disorders are important in improving peoples' standard of living and reducing the burden of mental disorders in society.

People with anxiety disorders have more comorbidities compared to those not suffering from anxiety. The most frequently reported anxiety comorbid conditions include cardiovascular disease,[3] diabetes,[4] thyroid illness,[5] and gastrointestinal disorders.[6] Meanwhile, anxiety disorders hinder the favorable prognosis of those diseases. The clinical manifestations of anxiety disorders are dominated by somatic symptoms, mainly dizziness, shortness of breath, chest pain, and palpitations.[7] Meanwhile, symptom profiles differ between men and women. Women with anxiety disorders more frequently experience physical discomfort, fatigue, and muscle tension compared to men.[8] Men with generalized anxiety disorder (GAD) are more likely to have alcohol and drug use disorders, whereas women have higher rates of severe comorbid mood problems.[9,10]

Anxiety and depression are closely related to each other, and they occur simultaneously. About 85% of patients with depression have significant anxiety, and 40% of patients with depression are also diagnosed with anxiety disorders.[11] As two of the most common psychiatric disorders, anxiety and depression share many similarities in terms of etiology, symptomatology, and even therapy. However,

anxiety and depression differ in terms of core symptoms and major pathogenic mechanisms. Typical causes of anxiety disorders include unexplained insecurity or worry, more of a fear of the future.[12] Anxiety disorders are believed to be caused by an imbalance between the inhibitory transmitter γ-aminobutyric acid (GABA) and the excitatory transmitter glutamate (Glu) in the brain. The main symptom of depression is a persistent low mood, a negative self-evaluation, and a sense of helplessness after suffering multiple social stresses in the past, and even suicidal tendencies in severe cases.[13] The recognized pathogenesis is deficiencies of monoamine neurotransmitters such as 5-hydroxytryptamine (5-HT) and norepinephrine (NE) in the synapse. Because of the severe suicidal tendency and the higher risk of depression, it needs to be excluded before diagnosing anxiety.

Currently, the exact pathogenesis of anxiety is still uncertain. It is generally believed that the occurrence of anxiety is associated with neurotransmitter dysfunction, neuroendocrine dysfunction, and immune-inflammatory activation. With the application of cutting-edge technological tools, some new potential pathological mechanisms of anxiety have been gradually discovered, such as ion channel dysfunction, excessive mitophagy in the amygdala, and neural circuit dysfunction. Considering that animal models are the most fundamental in the research on new drug exploitation and pathogenesis, this review presents the existing animal models of anxiety and some latest pathogenic mechanisms to provide directions for anxiety disorder therapy.

## 2 | CURRENT ANIMAL MODELS OF ANXIETY

### 2.1 | Unconditioned models

#### 2.1.1 | Predator stress model

The experimental design involves exposing rats to visual, olfactory, and acoustic stimuli associated with the predators while prohibiting physical interaction or attack.[14] Rats present anxiety-like behaviors such as fleeing around, crouching, rigid movements, and significantly increased excrement due to predator aggression or odors.[14] Acute predator stress and chronic predator stress upregulate the expression of corticotrophin-releasing hormone (CRH) mRNA, suggesting that predator stress induces anxiety behaviors by activating the hypothalamus–pituitary–adrenal axis (HPA axis).[15]

#### 2.1.2 | Social isolation model

Rodents have group-living characteristics. If rodents are forced to live alone, they will develop various adverse emotional and cognitive problems. Socially isolated for 3 weeks beginning on postnatal day (PND) 21 followed by 2 weeks of resocialization results in altering 5-HT release in the nucleus accumbens (NAc).[16] In addition, social isolation decreases baseline dopamine (DA) levels in the basolateral amygdala (BLA) and increases BLA excitability.[17]

#### 2.1.3 | Neonatal maternal separation model

Adverse early-life experiences are one of the major risk factors for developing mental illness in later life. Neonatal maternal separation is considered to be one of the models of early-life stress. Test protocols typically involve separating the pups from the dam for 3 h per day during the critical postnatal period. Alternatively, the pups are separated for 11 h on PND 24.[18] Neonatal rodents that undergo maternal deprivation exhibit more anxiety-like behaviors compared to the control group.[19] Studies have shown that maternal deprivation enhances the retention of fear memory and the late phase of long-term potentiation, and increases anxiety-like behaviors.[20]

### 2.2 | Conditioned models

#### 2.2.1 | Restraint stress

Restraint stress is currently the most common model of anxiety. Depending on the duration of restraint, it can be categorized into acute restraint stress (ARS) and chronic restraint stress (CRS).[21] ARS refers to a restraint stimulus that is given only once and lasts for a relatively short period.[21] CRS refers to a restraint stimulus that is repeated at regular consecutive intervals. In the elevated plus-maze (EPM) test, males exposed to CRS for 11 days showed higher anxiety-related behaviors than males exposed to CRS for 22 days, but the same behavior was not observed in females.[22] Meanwhile, chronic stress impairs hippocampal function as evidenced by poor spatial ability in male rats but not in females.[23] It is possible that females are more resistant to the harmful effects of restraint stress on anxiety.

#### 2.2.2 | Fear-conditioning anxiety model

Conditioned fear combines certain neutral conditioned stimulus (sound, light, and environment) with an aversive unconditioned stimulus (foot shock) to elicit a conditioned reflex in the animal. It is followed by a neutral conditioned stimulus given alone to induce fear and anxiety caused by the aversive conditioned fear.[24] Based on this theory, the conditioned fear model commonly used in animal models of anxiety generally combines sound signals with electrical stimulation signals, and then emits the sound signal alone to trigger anxiety response in animals.[25]

#### 2.2.3 | Repeated social defeat

Most anxiety-related stimuli possess social features, including environmental stress, life events, and interpersonal frustrations. Repeated social defeat (RSD) simulates the process of social factors contributing to anxiety. In the RSD model, an aggressive intruder male CD-1 mouse is introduced in cages of established male cohorts of mice for

six consecutive nights, lasting for 2 h per day.[26] They are observed to ensure that the resident mice exhibit subordinate behavior.

### 2.3 | Drug models

Administering drugs alters the levels of hormones and neurotransmitters in animals, disrupting the normal mechanism and inducing anxiety. Common models include caffeine-induced anxiety, lipopolysaccharide (LPS)-induced anxiety, and 5-HT-induced anxiety.

Studies have demonstrated that caffeine intake activates calcium channels and increases anxiety-like behaviors. Therefore, this model can apply to the screening of antianxiety novel agents for the L-type Ca channel.[27] The LPS-induced model simulates anxiety induced by dysbiosis of the gut microbiome. It is commonly available for studies on the etiology of anxiety and the exploitation of probiotics.[28] The 5-HT-induced model alters 5-HT and NE levels in the synaptic gap to build an anxiety model, applied in screening for antianxiety novel agents that act on the 5-HT system and the NE system.[29]

### 2.4 | Genetic models

Anxiety disorders are moderately heritable. Therefore, altering the expression of genes associated with anxiety is a method to build anxiety models. One of the modeling approaches is selective breeding. Based on the sensitivity to stress, animals with a higher sensitivity to stress response are selected for breeding.[30] Based on the assumption that the 5-HT system is dysfunctional in anxiety disorders, mice lacking the gene that codes for the serotonin transporter and 5-HT1A receptors exhibit increased anxiety-like behaviors due to impaired serotonin reuptake.[31,32] Meanwhile, mice with genetic deletion of GABAAR or GABABR increase anxiety-like behaviors due to reduced neuronal inhibition.[33] Additionally, the HPA axis is an important part of the stress response. It has been demonstrated that CRH receptor 1 knockout mice exhibit reduced anxiety-like behaviors,[34] in contrast to CRH receptor 2 knockout mice.[35]

## 3 | BEHAVIORAL TESTS

Exploratory behavioral models are available for the identification and assessment of anxiety states, applied to evaluate the success of anxiety modeling. Those tests are mostly in conjunction with each other to assess anxiety behaviors.

### 3.1 | EPM test

The EPM uses the rodent's inquisitive mind for novel environments and fear of high open arms to form a conflict to simulate an anxiety state,[36] for example, placing the animal in the center of the maze so that its head faces the closed arm and observing its activity after release. The evaluation indexes are the percentage of open arm/total entries (OE%) and the percentage of open arm/total time (OT%).[37]

### 3.2 | Open field test

The open field test (OFT) is a method to evaluate the autonomous behavior, exploratory behavior, and tension of experimental animals in a novel environment. The experiment is performed in a plain open field arena with the bottom surface divided into square grids of equal size, the interior walls and bottom squares blackened, the squares along the walls as the peripheral area, and the rest as the central area.[38] The evaluation indicators are center distance, center time, corner time, wall time, and number of excrements.[39]

### 3.3 | Light–dark exploration test

The light–dark exploration test (LDT) is based on a conflict between the psychology of rodents' fear of bright places and their preference for exploration. The experimenter places the animal inside the shuttle box arch and records the behavioral changes of the animal within 5 min.[25] The evaluation indicators are the percentage of time spent in light, the percentage of horizontal movements in light, the percentage of vertical movements in light, and the number of shuttles.[40] It has been demonstrated that antianxiety drugs increase the number of times the animal shuttles through the box and the duration of stay in the light.[41]

### 3.4 | Elevated zero maze test

The elevated zero maze test is an improved version of the EPM test with the advantage that the exploration can be continuous, unaffected by the terminals of the arms, and without a central zone.[42] The device is an elevated circular platform consisting of successively alternating two opened and two closed areas. Rodents are placed in the center of one of the open areas to freely explore the maze for a total duration of 5 min.[43] The evaluation indicators are the number of rodents probing the edge of the platform, percentage of open areas/total time, and the number of entries into the open areas.[44]

### 3.5 | Novelty-suppressed feeding test

The novelty-suppressed feeding test (NSFT) utilizes the animal's desire to feed in a hungry state and the fear of entering the central bright area to form a paradoxical conflict to inspect the animal's anxiety state.[42] In brief, the animals are not fed 24 or 48 h before testing, and only water is provided. During testing, the animals are placed in one corner of the field arena.[45] Three food pellets are placed in the center of the arena. The evaluation indicators are the feeding latency and the amount of food consumed within 5 or 10 min (Table 1).[46]

TABLE 1 Advantages and disadvantages of animal models of anxiety.

| Anxiety modeling approaches | | Advantages | Disadvantages |
|---|---|---|---|
| Unconditioned models | Predator stress model | 1. Similar to the clinical features of anxiety disorder<br>2. Less harm to animals<br>3. Less time consuming | 1. Pollute the laboratory<br>2. Stimulation intensity cannot be controlled |
| | Social isolation model | 1. Simulate the psychological stress<br>2. Simple experimental operation | 1. Can be confused with depression |
| | Neonatal maternal separation model | 1. Simulate the psychological stress | 1. Many uncertainties affect the experimental results |
| Conditioned models | Rrestraint stress | 1. Simulate the adverse emotional and somatic responses | 1. Limited to somatic stress only<br>2. Can be confused with depression |
| | The fear-conditioning anxiety model | 1. Persistent behavioral changes | 1. Many factors affect the experiment, which adds much uncertainty to the modeling results |
| | Repeated social defeat | 1. Simulate anxiety disorder due to social factors<br>2. Lasting behavioral and biological changes | 1. Mice are susceptible to injury, which affects assessment results<br>2. Can be confused with depression |
| Drug models | | 1. Simple experimental operation<br>2. Preliminary screening of antianxiety drugs | 1. Short duration<br>2. Single channel<br>3. Drug dose and dosing cycle have a large impact on the outcome<br>4. Differences exist between rodents and humans |
| Genetic models | | 1. For the study of molecular mechanisms of diseases and gene therapies of diseases | 1. Costly<br>2. Difficult |
| Behavioral tests | | | |
| Elevated plus-maze test<br>Open field test<br>Light/dark exploration test<br>Elevated zero maze<br>Novelty-suppressed feeding test | | 1. Simple experimental operation<br>2. Less harm to animals<br>3. Intuitive response to animal conditioned response<br>4. Semi-automation makes the results more objective and realistic | 1. Time consuming<br>2. OFT and NSFT can be confused with depression |

Abbreviations: NSFT, novelty-suppressed feeding test; OFT, open field test.

## 4 | THE PATHOGENESIS OF ANXIETY

### 4.1 | Neurotransmitter dysfunction

Neurotransmitters are a class of biologically active chemicals in the nervous system. Information transfer takes place mainly through chemical synapses. Dysfunction of neurotransmitters in the synaptic gap affects information transmission and may trigger anxiety.

#### 4.1.1 | γ-aminobutyric acid

GABA (γ-aminobutyric acid), a naturally occurring nonprotein amino acid, is an important inhibitory neurotransmitter in the mammalian central nervous system (CNS). Evidence from clinical studies suggests that GABA levels are generally low in the serum and brain of patients with anxiety disorders.[47] Functional studies have reported that GABA release from NG2 glial cells affects inhibitory synapses in proximal interneurons and reduces GABA output from interneurons, ultimately causing an excitatory–inhibitory imbalance in local neural microcircuits in the hippocampus to induce anxiety-like behaviors.[48,49] Furthermore, amygdala hyperactivation is an important process in the development of anxiety. Loss of GABAergic interneurons or reduction in glutamate decarboxylase may lead to the reduced presynaptic release of GABA followed by BLA hyperexcitability and consequently increased anxiety levels.[50,51]

#### 4.1.2 | Glutamate

Glu is an excitatory neurotransmitter that plays an important role in message transmission. The brain functions optimally by requiring a balance between excitation and inhibition. Glutamine synthesizes Glu through a series of reactions, which is decarboxylated to synthesize GABA, forming the glutamine-Glu/GABA cycle.[52] When this cycle suffers from dysfunction, the Glu–GABA balance in the body breaks down and leads to the onset of anxiety. Studies have found significantly higher levels of glutamate and glutamine and lower levels of GABA in the brains of patients with social anxiety disorder.[53] It has also been found that the Glu/GABA ratio is proportional to the degree of anxiety in animal models,[54] suggesting that high Glu levels induce the onset of anxiety. In addition, Glu

transporter EAAT2 is responsible for most of the Glu transport and uptake. EAAT2 dysfunction leads to high Glu concentrations in the synaptic gap that affects the onset and development of anxiety.[55]

#### 4.1.3 | 5-Hydroxytryptamine

5-hydroxytryptamine neurons and receptors in the brain, mainly located in the limbic system, hippocampus, septal nucleus, and septal nucleus, are involved in mood regulation. Clinical studies have found that 5-HT synthesis was temporarily reduced with acute tryptophan depletion, and anxiety scores were increased in healthy subjects.[56] Postadministration of a 5-HT2CR agonist exerts antianxiety effects,[57] whereas the agonist injected in the NAc (BNST [bed nucleus of the stria terminalis]) causes anxiety by activating postsynaptic receptors.[58] It is suggested that 5-HT2C receptors in different brain regions modulate anxiety differently. It has been shown that optogenetic inhibition of 5-HT input to the dorsal bed nucleus of the stria terminalis increases anxiety-like behaviors, which is mediated through 5-HT1A receptors.[59,60] Activation of 5-HT2C receptors throughout the BNST is known to evoke anxiety-like behaviors,[61] whereas activation of 5-HT1A receptors is antianxiety,[58,59] suggesting that differential distribution of high-affinity 5-HT1A receptors and low-affinity 5-HT2C receptors affects the ultimate effect of 5-HT on brain regions.

#### 4.1.4 | Dopamine

DA is the most abundant catecholamine neurotransmitter in the brain. Clinical studies have shown that upregulation of NAc/vPAL (the bilateral ventral pallidum) D2 and D3 receptors on the left side and increased seven-item GAD questionnaire scores are found to be significantly associated with higher anxiety symptoms.[62] Intra-amygdala infusion of dopamine D1/2 receptor agonists resulted in increased anxiety-like behavior in rats,[63] but systemic administration of dopamine agonists reduced anxiety-like behavior.[64] It has shown that dopamine-depleted neonatal rats have antianxiety effects in adulthood, with reduced sensitivity to changes in the intensity of anxiety-provoking stimuli.[65] Also, in the striatum of dopamine-depleted neonatal rats, enhanced activity of 5-HTergic neurons and increased expression of 5-HT2A receptors were observed, which may be one of the pathways for its antianxiety effect.

#### 4.1.5 | Noradrenaline

NE neurons are more centrally distributed in the brain, with the vast majority located in the midbrain reticular formation, the blue spot of the pons, and the ventral–lateral portion of the medulla oblongata. Studies have shown higher serum concentrations of NE in patients with GAD and panic disorder (PD).[66] The locus coeruleus (LC) contains the largest number and highest density of noradrenergic cell bodies in the brain. During stressful conditions, LC-NE neurons are activated to promote the release of NE and normalize message transmission.[67] Conversely, after the stress experience, the neuronal firing rate in the LC increases, resulting in increased NE concentrations in peripheral blood and upregulation of tyrosine hydroxylase and dopamine β-hydroxylase expression in the central nervous tissue, and thus results in anxiety.[68] The receptors for NE are mainly divided into α- and β-adrenergic receptors. Studies have revealed that activation of α1 and β receptors is anxiogenic, and activation of α2 receptors produces antianxiety effects.[69,70]

#### 4.1.6 | Acetylcholine

Acetylcholine (Ach) in the brain alters neuronal excitability, affects synaptic transmission, induces synaptic plasticity, and coordinates the firing of neuron groups. ACh receptors consist of two primary members, namely muscarinic acetylcholine receptors (mAChRs) and nicotinic acetylcholine receptors (nAChRs). Choline is one of the substances that synthesize ACh. Plasma concentrations of choline have been negatively correlated with anxiety symptoms in a large-scale population-based study.[71] It has been revealed that CRS increases the expression of mAChR in the ventral hippocampus (vHPC) of mice and vHPC ACh release in mice, inducing social avoidance and anxiety-like behaviors.[72] Studies have demonstrated that activation of nAChRs and mAChRs induces anxiety-like behaviors and that knockdown or inhibition of nAChRs and mAChRs has antianxiety effects.[73]

#### 4.1.7 | Neuropeptide Y

Neuropeptide (NPY), a peptide consisting of 36 amino acid residues, is widely distributed in the central and peripheral nervous systems. Increased anxiety-like behaviors of rats are accompanied by elevated levels of NPY in the prefrontal cortex (PFC), frontal cortex, cingulate cortex, striatum, and periaqueductal gray matter of the midbrain.[74] Similarly, expression levels of anxiety-associated genes and mRNA levels of cortical-like receptor genes are significantly higher in NPY gene-deficient zebrafish than in wild-type fish.[75] It has shown that the administration of NPY in the dorsal hippocampus, dorsolateral septum, central amygdala (CeA), and BLA had antianxiety-like effects, with no significant changes when administered to the medial amygdala (MeA).[76] NPY Y1 and Y2 receptors play an important role in mood regulation. Most studies have supported that activation of Y1 receptors has antianxiety effects and activation of Y2 receptors has anxiogenic effects.[77,78]

### 4.2 | Neuroendocrine dysfunction

Stress reactions from external environmental stimuli trigger dysfunction of the neuroendocrine system. And prolonged stress reactions can trigger anxiety.

### 4.2.1 | Renin-angiotensin system

The renin-angiotensin system (RAS) is present in the circulatory system, maintaining the relative stability of the extracellular fluid. Clinical statistics have indicated that the I/D polymorphism of the angiotensin-converting enzyme (ACE) gene and the T/C polymorphism of the angiotensinogen gene are more common in men with PD.[79] The octopeptide angiotensin II acts mainly through two G-protein-coupled receptors, AT1 and AT2. Inhibition or downregulation of AT1 receptors inhibits the response of the HPA axis to stress and promotes the binding of cortical corticotropin-releasing factor type 1 receptors and benzodiazepines.[80] It has also been demonstrated that stress decreases the expression of AT2 receptors in the brain and that inhibition or knockdown of AT2 receptors evokes anxiety-like behaviors.[81] Angiotensin-(1–7) (Ang-(1–7)) is a counter-regulatory peptide of the RAS. Transgenic rats overexpressing Ang-(1–7) exhibit less anxiety-like behavior, which increases again after input of Mas receptor antagonists.[82] Increased angiotensin-converting enzyme 2 (ACE2) activity has antianxiety effects accompanied by Mas receptor activation.[83] Activation of ACE2, Ang-(1–7), and Mas receptor has antianxiety effects, and Mas receptor activation may be one of the ACE2 and Ang-(1–7) antianxiety pathways (Figure 1).

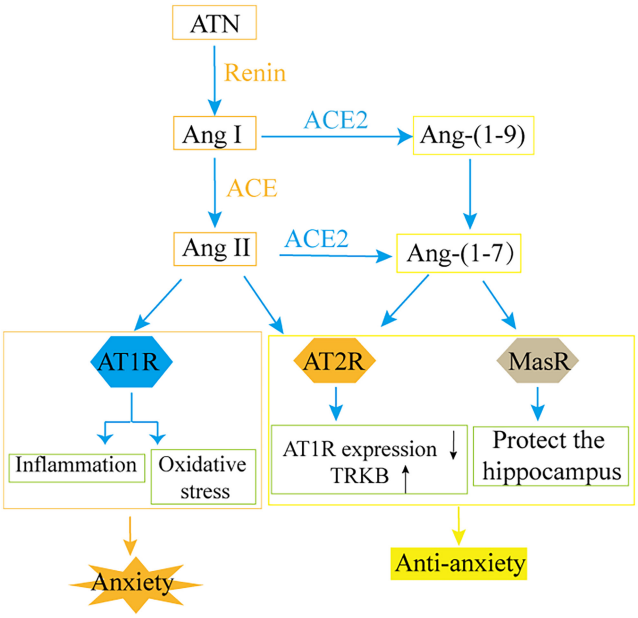


**FIGURE 1** A summary of RAS (renin-angiotensin system) effects on anxiety. The RAS comprises two axes: the classical axis (ACE [angiotensin-converting enzyme]/Ang II [angiotensin II]/AT1R) and the nonclassical axis (ACE2 [angiotensin-converting enzyme 2]/Ang-(1–7) [angiotensin-(1–7)]/MasR). Under normal physiological conditions, the two pathways regulate each other and maintain a dynamic balance. Ang II exacerbates oxidative stress and inflammatory responses by activating the AT1R to upregulate the ACE/Ang II/AT1R pathway, which promotes the onset of anxiety disorders. Ang II activates the nonclassical pathway by activating the AT2R and MasR to achieve anxiolytic effects.

### 4.2.2 | HPA axis

The HPA axis is an important part of the neuroendocrine system, involved in controlling stress responses and regulating physical activity. Numerous investigations have demonstrated that patients with anxiety disorders have high levels of adrenocorticotropic hormone (ACTH) and glucocorticoid (GC) and an overall hyperactive HPA axis.[84] Under normal conditions, corticotropic hormones secreted by the adrenal glands provide negative feedback regulation of the pituitary and hypothalamus.[85] Under stressful situations, stress disrupts the negative feedback regulation mechanism and leads to continuous activation of the HPA axis and continuous production of GC, producing anxiety effects (Figure 2).[86] Therefore, appropriate inhibition of the HPA axis will attenuate the onset of anxiety.

In the hypothalamic–pituitary–thyroid (HPT) axis, hypothyroidism decreases triiodothyronine (T3) level in the hippocampus, reduces the expression of the T3-dependent gene *Dio3* in the amygdala, downregulates the *Calb2* gene encoding the calcium-binding protein which is associated with GABAergic transmission, and increases anxiety-like behaviors in mice.

### 4.2.3 | HPT axis

The HPT axis has great significance for nerve cell excitability, transmitter regulation, and central neurodevelopment. Susanne Fischer et al.[87] have found the highest prevalence of comorbid thyroid disease in patients with GAD using comorbidity analysis, and most patients exhibit hypothyroidism. They have shown that rats under

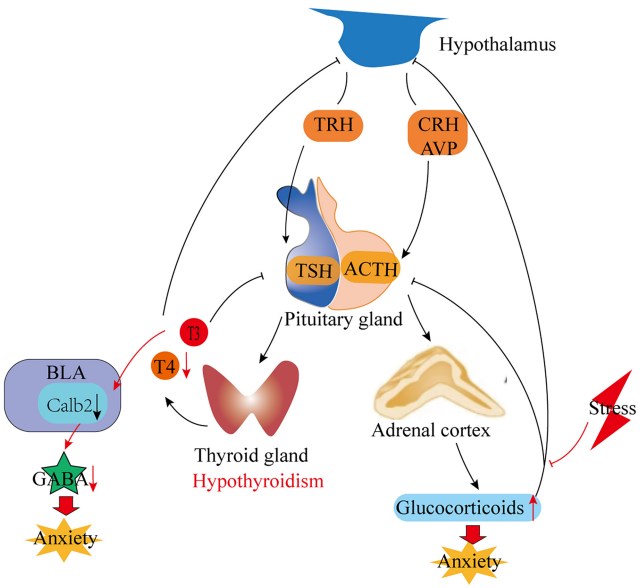


**FIGURE 2** In the HPA (hypothalamus–pituitary–adrenal) axis, stress disrupts negative feedback regulation during anxious situations and leads to continuous activation of the HPA axis and constant glucocorticoid production, resulting in anxiety.

stress, with lower levels of T3 and tetraiodothyronine in peripheral serum, are more vulnerable to anxiety than normal rats.[88] Evidence supports that hypothyroidism induces anxiety. T3 concentration can be regulated by type 2 deiodinase. In D2 knockout mouse brains, T3 levels were reduced in the hippocampus, and the expression of the T3-dependent gene *Dio3* was lower in the amygdala. Meanwhile, the *Calb2* gene encoding the calcium-binding protein associated with GABAergic transmission was downregulated, and anxiety-like behaviors increased in mice (Figure 2).[88]

#### 4.2.4 | Hypothalamic–pituitary–gonadal axis

The hypothalamic–pituitary–gonadal (HPG) axis primarily regulates the secretion of sex hormones in humans. Studies have found that children in adolescence and adults in menopause are more prone to anxiety.[89,90] Gonadotropin-inhibitory hormone (GnIH) is an inhibitor of the HPG axis. Experiments have shown that intracerebroventricular injection of GnIH in male rats induces anxiety-like behaviors.[91] The amygdala, BNST, vHPC, and PFC are considered to be key brain nuclei in the control of fear and anxiety, and they are interconnected. Dense GnIH-ir fibers are found in this interconnected circuit, so GnIH neurons may cause anxiety by inhibiting interconnected neurons in the lateral septum (LS), BNST, and MeA.[92]

### 4.3 | Immune dysfunction

Patients with PD were found to have remarkably higher levels of C-reactive protein, interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α).[93] Statistical analysis has revealed that the anxiety state positively correlates with the IFN-γ/IL-4 (anti-inflammatory cytokine interleukin 4) ratio.[94,95] Microglia are immune cells of the nervous system and are involved in regulating the progression of the nervous system as well as in maintaining neurological homeostasis. Microglia recruit monocytes to the brain during stress, and these inflammatory monocytes upregulate the pro-inflammatory cytokine interleukin-1β and brain endothelial interleukin-1 receptor type 1 expression to promote anxiety.[96,97] During inflammatory responses in the brain, the expression of pro-inflammatory cytokines such as TNF-α in the brain can stimulate the HPA axis, which leads to elevated levels of ACTH and cortisol, triggering anxiety.[98]

### 4.4 | Brain-derived neurotrophic factor

Brain-derived neurotrophic factor (BDNF) is the most abundant neurotrophic factor in the brain, which can repair damaged neurons and is closely related to messaging. Studies have revealed that plasma concentrations of BDNF in patients with GAD are significantly lower than those in the control group.[99] The main site of action of hippocampal BDNF is the infralimbic medial prefrontal cortex (IL-mPFC). Infusing BDNF into the IL-mPFC and hippocampus reduced fear.[100] The absence of BDNF may disrupt the processing of fear memory by the hippocampal-IL mPFC pathway, which induces the onset of anxiety.[101] In addition, different forms of BDNF produce different effects on anxiety. Pro-BDNF induces neuronal apoptosis via P75 NTR receptors, whereas mature BDNF can inhibit apoptosis via TrkB receptors to protect neurons.[102] This is demonstrated in several studies that GCs interact with endogenous BDNF–TrkB signaling to enhance the expression of fear memory.[103,104] Furthermore, the valine$^{66}$methionine (Val$^{66}$Met) polymorphism (rs6265) of the BDNF gene has been demonstrated to modulate stress susceptibility and stress-inducible neuropsychiatric endophenotypes. Compared with Met allele carriers, Val/Val homozygotes exhibited significantly blunted vagal withdrawal and vagal activation, which increases the risk of anxiety disorders.[105,106]

### 4.5 | Neuronal plasticity

A decrease in the number of neurons and glial cells in the brain of stressful and anxious individuals was found, with significant changes in glial cells.[107,108] In proapoptotic gene Bax-knockout mice, increased adult neurogenesis not only reduced anxiety- and depression-related behaviors in mice administered GCs for a long time but also counteracted the effects of unpredictable chronic mild stress and endocrine levels.[109] Stress and anxiety also decrease hippocampal neuron numbers, and low neurogenesis fails to mitigate stress and can further exacerbate anxiety.[110] Furthermore, material and branch points on apical dendrites are significantly reduced in the hippocampus and PFC in animal models of anxiety, especially in the high-anxiety group.[111] Loss of dendrites contributes to animals being more sensitive to stress and more prone to anxiety.[112]

### 4.6 | Ion channels

Ion channels generate and transmit electrical signals that are directly related to cellular excitability and are a common basis for arousability in different tissues. Expression of ion channels has been linked to messaging and the occurrence of anxiety-like emotions and behaviors. It has been demonstrated that CRS leads to the loss of small-conductance calcium-activated potassium channel 2 (SK2)-mediated currents that exacerbate anxiety-like behaviors.[111] Similarly, SK2 expression in BLA alleviates anxiety.[113] The speculated mechanism is that SK2 channel activation causes membrane hyperpolarization, which decreases neuronal excitability.[114] Acid-sensing ion channel 1a (ASIC1a) is abundantly expressed in the amygdala complex and other brain regions associated with fear. Overexpression of ASIC1a increased anxiety-like behaviors, whereas knockdown of ASIC1a relieved anxiety.[115] In addition, the possibility that modulation of T-type calcium channels may aggravate or improve anxiety-related behaviors suggests a dual role for these channels.[116,117]

### 4.7 | Endocannabinoid system

The endocannabinoid system (ECS) is one of the crucial regulatory systems in the CNS that modulates the neuroinflammatory response, maintains immune homeostasis, and is involved in regulating various physiological functions such as stress, learning, memory, pain, and mood. There are two main endocannabinoids: *N*-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG), both synthesized and released from postsynaptic terminals, transmitting retrograde signals.[118] Fatty acid amid hydrolase (FAAH) and monoacylglycerol lipase (MAGL) are the corresponding hydrolases. It has been found that increased FAAH activity and decreased AEA signaling increase anxiety-like behaviors.[119,120] Acute 2-AG depletion increased anxiety-like behaviors, whereas elevating 2-AG levels with MAGL inhibitor alleviated anxiety in mice and reduced anxiety-like behaviors induced by AEA deficiency.[121] Cannabinoid receptors consist of two primary members, namely CB1R and CB2R, the former primarily involving neuromodulation and the latter focusing on immunomodulation.[122] Studies have shown that the appropriate activation of CB1R reduces anxiety-like behaviors.[123] Activation of CB2R prevents the increase in stress-induced inflammatory factors (Figure 3).[124]

### 4.8 | Neural circuits

Anxiety is mediated by interactions between different parts of the same brain regions as well as between different brain regions. Several brain areas related to emotion regulation, such as the amygdala, mPFC, vHPC, and BNST, are interconnected to form multiple neural circuits mediating anxiety (Figure 4).[125]

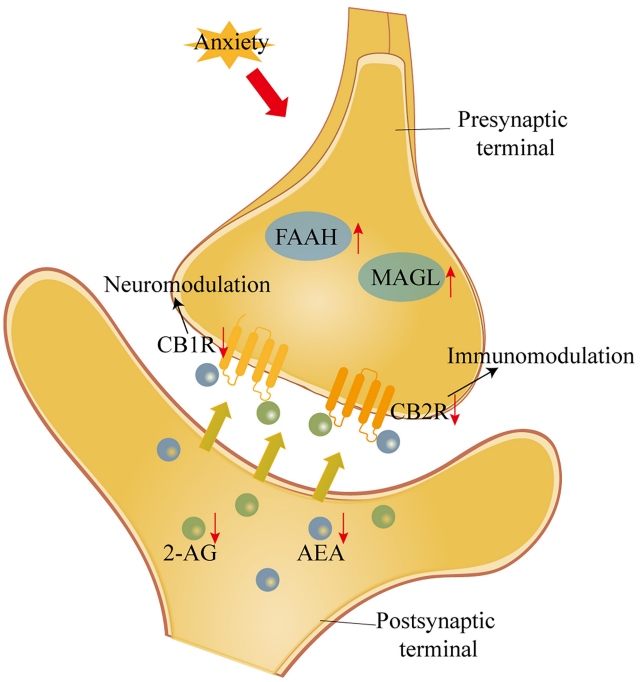


FIGURE 3 Mechanism of association between ECS (endocannabinoid system) and anxiety disorders.

The amygdala is the central region of the limbic system of the brain. It plays an important role in the regulation of anxiety, fear-related behaviors, physiological activities, and hormone levels. The BLA, which receives external information input, and the CeA, which is responsible for information output, are particularly important in anxiety processing.[126,127] The connections between the nucleus groups of the amygdala, as well as various afferent or efferent amygdala projections, have different functions in the developmental process of anxiety.[128] Different output pathways of the BLA reinforce or inhibit anxiety-like behaviors. In optogenetic studies, BLA projections to CeA manifested antianxiety effects in EPM and OFT.[129] BLA projections to the mPFC decreased social interactions and increased anxiety-like behaviors.[130] BNST, a component of the extended amygdala, is involved in the modulation of anxiety-related neural circuits controlling defense-related behavioral responses (Figure 4). Moreover, activating the insular cortex, BLA, is anxiolytic,[131] and activating the insular cortex–paraventricular thalamus–BNST neural circuit increased the susceptibility of stress-induced anxiety-related behaviors.[132]

Neural inputs are considered to be the major components of the anxiety circuit from the vHPC to the lateral hypothalamic area, the LS, and the mPFC. However, the exact mechanism of action is still not fully understood. In vivo experiments have found that activation of LS-projecting vHPC cells reduces anxiety and inhibition of the same cell populations increases anxiety.[133] In contrast, activation of mPFC-projecting vHPC cells increased anxiety-like behaviors.[133,134] Thus, it is speculated that the regulation of anxiety by vHPC is mediated by a dynamic balance between cells projecting onto the LS and mPFC.

### 4.9 | Gut microbiota

A growing body of evidence supports that the microbiome and microbiota-derived molecules play an important role in the regulation of behaviors associated with psychiatric disorders. A randomized controlled trial of probiotic supplementation through capsules significantly improved neurophysiological anxiety in participants.[135] The gut microbiota can affect the synthesis and metabolism of neurotransmitters, which then act on the enteric nervous system to send signals to the brain via the vagus nerve to affect mood modulation.[136,137] Gut peptides are essential regulators of microbiota–gut–brain signaling in health- and stress-related psychiatric disorders, such as glucagon-like peptide, peptide YY, cholecystokinin, corticotropin-releasing factor, oxytocin, and ghrelin. The signaling of these gut peptides affects the regulation of anxiety.[138,139]

### 4.10 | Mitophagy

An increasing number of studies have shown that excessive mitophagy induces anxiety. PINK1 (phosphatase and tensin homologue-induced putative kinase 1) and Parkin (an E3 ubiquitin ligase) are two key proteins involved in mitophagy. It has been shown that

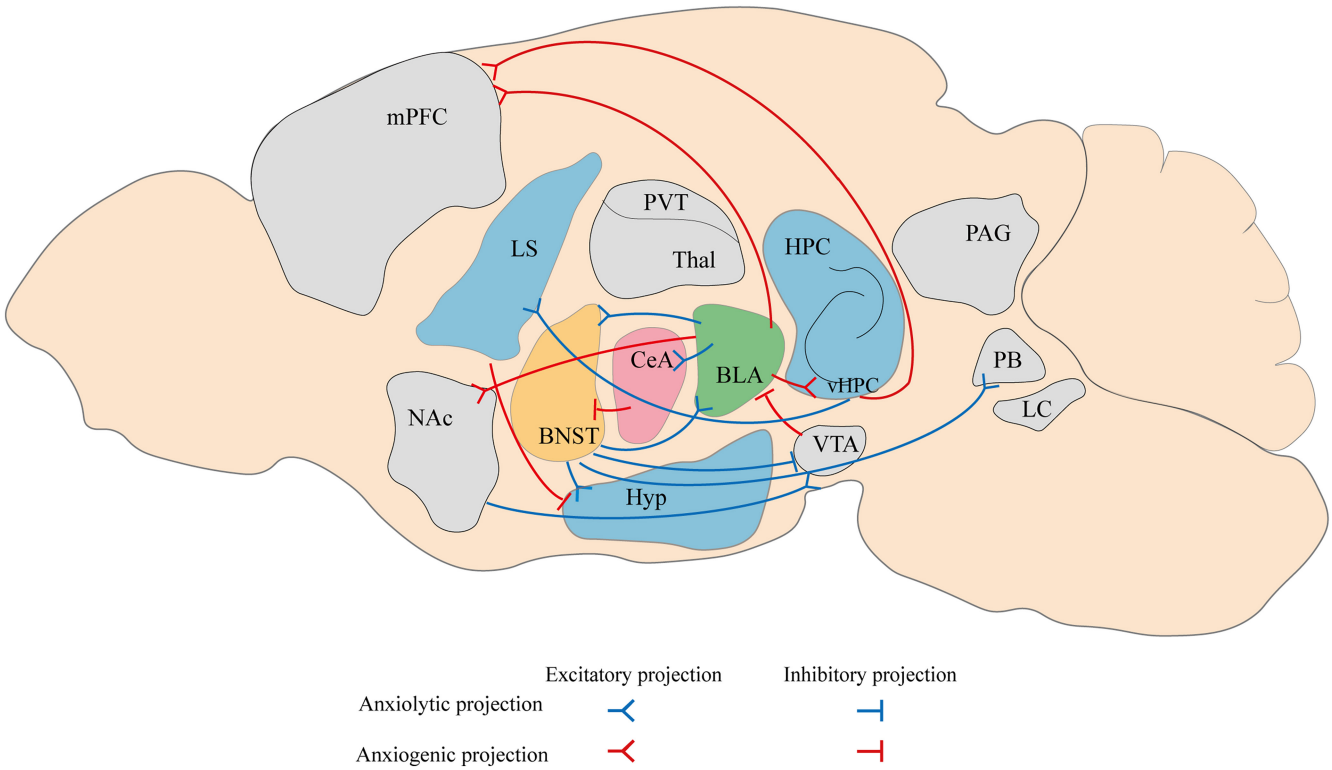


FIGURE 4 Anxiety neural circuitry. Anxiety states are mediated by abnormal neural projections between multiple brain regions. BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central amygdala; HPC, hippocampus; Hyp, hypothalamus; LC, locus coeruleus; LS, lateral septum; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; PAG, periaqueductal gray; PB, parabrachial nucleus; PVT, paraventricular thalamus; Thal, thalamus; vHPC, ventral hippocampus; VTA, ventral tegmental area.

chronic social defeat stress in mice leads to mitochondrial damage, which selectively triggers the PINK1–Parkin mitophagy pathway in the amygdala, and consequently attenuates synaptic transmission in the BLA-BNST antianxiety pathway, leading to worsening anxiety.[140] Furthermore, excessive mitophagy inhibits the growth and development of hippocampal neurons, which affects information processing and transmission, and thus affects the regulation of emotions in the hippocampus.[141,142]

### 4.11 | Genetic factors

Twin studies have suggested that anxiety disorders are moderately heritable. Genetic epidemiological studies report the immediate family members of a patient with anxiety disorders have a four- to six-fold increased risk of the disease.[143] The heritability is estimated to be 30%–50%.[143] Anxiety disorders are considered to be highly polygenic. The degree of gene expression is associated with the severity of the anxiety disorder, especially genes relevant to monoaminergic neurotransmission or stress axis function.[144,145] DNA methylation, histone modification, and noncoding RNA regulation play important roles in epigenetic mechanisms of anxiety disorders.[146,147] Unlike DNA sequence changes, the processes involved in the regulation of gene transcription and expression are reversible. Histone deacetylase inhibitors have shown a positive effect on both preclinical and clinical drug trials for the treatment of anxiety disorders, providing new insights and directions for epigenetic pharmacotherapy of anxiety disorders.[148] Furthermore, an increasing number of studies have demonstrated that anxiety results from gene–environment interactions: individuals with high genetic susceptibility show more anxious behaviors under stress,[149] and individuals who are frequently under stress also have increased variation in anxiety-related genes.[150]

## 5 | SUMMARY AND PROSPECTS

Animal models of anxiety partially simulate the symptoms observed in patients with anxiety disorders. However, the majority of anxiety modeling relies on somatic stress methods, and animals tend to exhibit trait anxiety tendencies. In clinical studies, the pathogenesis of anxiety disorders is mostly attributed to psychological stress and state anxiety, which differs from the model. The drug-induced model focuses on the currently widely recognized pathogenesis of anxiety, mainly used for the initial screening of antianxiety novel agents. However, there are differences in neurotransmitter systems and signaling pathways between rodents and humans.[151] Consequently, the effects observed in experiments might not align with clinical effectiveness, posing a challenge when transitioning from animal studies to clinical trials. Gene knockout will affect many compensatory and downstream pathways, making it difficult to conduct studies on

pathogenic mechanisms. Therefore, genetic models are frequently employed for investigating molecular mechanisms and genetic factors. Anyway, various animal models of anxiety have different pathological and physiological mechanisms. Meanwhile, gender, strain, and individual differences and the experimental environment vary significantly. Therefore, it is difficult to avoid false-positive results. Contrary to the findings of clinical studies, some female mice have exhibited less anxiety-like behavior.[152,153] Moreover, using a single method can produce only a certain aspect of symptoms of anxiety. Therefore, a successful modeling approach should encompass various methods to enhance both feasibility and model accuracy.

Anxiety states are typically evaluated relative to control conditions, yet there are no universally accepted criteria for determining model success. Behavioral evaluation measures of anxiety models prefer different methods. As expected, EPM, OFT, and LDT are commonly combined to gauge anxiety levels, although the interplay between these behavioral tests should also be taken into account. In addition, OFT and NSFT are regularly used to estimate depression and anxiety levels in animals. However, there is no method to clearly distinguish between depression and anxiety. Evaluation indicators are indispensable for the reliability and accuracy of the model.

The pathogenesis of anxiety disorders is extremely complex and coinduced by gene, environment, and physiological imbalances. Nevertheless, the status of each causative factor in the pathogenesis needs to be further elucidated. Understanding the links between the various hypotheses is also an important part of the investigation of the pathogenesis of anxiety. Current research suggests that pro-inflammatory factors are increased and immune inflammation is activated in anxiety states. Meanwhile, pro-inflammatory factors can trigger the HPA axis, affecting neurotransmitter synthesis, release, and reuptake, which is also closely related to the stability of the gut microbiome.[97,98,154] Moreover, neuroplasticity is an important potential target for the pathogenesis of anxiety nowadays. Neuroplasticity is associated with neurotrophic factors, GC levels, and immune inflammation.[109,155] Therefore, a holistic understanding of the links between mechanisms also provides new ideas for the exploration of the pathogenesis of anxiety.

In conclusion, the establishment of anxiety animal models is an indispensable part of the pathogenetic mechanism research and new drug development process. As drug development and the pathophysiology of anxiety have been intensively studied, the stability and reproducibility of animal models have also been improved. The current research status is discussed to develop more efficient treatments for targeted patients.

## AUTHOR CONTRIBUTIONS

Hongqing Zhao: conceptualization, writing of the original draft, and writing—reviewing and editing. Mi Zhou: conceptualization, writing of the original draft, and writing—editing. Yang Liu: writing—review and editing. Jiaqi Jiang: writing—review and editing. Yuhong Wang: supervision and writing—review and editing. All authors approved the final version of the manuscript for submission.

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## CONFLICF OF INTEREST STATEMENT

The authors declare no conflict of interest.

## EHICS STATEMENT

Our study did not require further ethics committee approval as it did not involve animal or human clinical trials and was not unethical. Following the ethical principles outlined in the Declaration of Helsinki, all participants provided informed consent before participating in the study. The anonymity and confidentiality of the participants were guaranteed, and participation was completely voluntary.

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