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Pathological Research and Target Exploration of Anxiety Disorders

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Abstract: Anxiety disorders are widespread mental health problems, impacting around 4% of the global population and showing a 7.57% lifetime prevalence among Chinese adults. These conditions are mainly marked by intense worry and tension without actual threats, presenting in various forms and symptoms. Abnormalities in the structure and function of certain brain areas, along with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, have been linked to the development of anxiety disorders. For instance, changes in brain structures like the amygdala and parahippocampal gyrus can disrupt the regulation of emotions and memory processing. The understanding of what causes anxiety disorders has evolved significantly over time. Although the serotonin hypothesis has faced increasing doubts, the 5-hydroxytryptamine (5-HT) system remains a crucial target for treatment. The 5-HT system consists of multiple receptor subtypes that control different physiological processes. Medications such as selective serotonin reuptake inhibitors (SSRI) and serotonin-norepinephrine reuptake inhibitors (SNRI) work by preventing the reuptake of neurotransmitters. Gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the brain, is essential for the synthesis, metabolism, and receptor activities related to anxiety disorders. Benzodiazepines relieve anxiety symptoms by adjusting GABAergic activity. Studies on the pathophysiology and treatment targets of anxiety disorders lay the groundwork for the development of new diagnostic methods and treatment approaches.

Keywords: Anxiety Disorder; Serotonin; γ-aminobutyric Acid; Mental Health; Psychiatric Diagnosis.

1. Introduction

In today's fast - changing society, anxiety disorders have become a critical global public health problem that urgently needs attention. These disorders have a far - reaching influence on both physical and psychological health, as well as the overall standard of living. Based on 2019 statistics from the World Health Organization (WHO), about 300 million people across the globe, including 58 million children and teenagers, are afflicted by anxiety disorders. In China, the incidence of anxiety disorders is remarkably high. The 2019 Epidemiological Survey of Mental Disorders Among Chinese Adults showed that such disorders rank first among seven major mental health conditions, with a lifetime incidence rate of 7.57% and an annual incidence rate of 4.98%, affecting around 40 million people every year [1]. Moreover, the 2022 National Mental Health Survey in the United States showed that the detection rate of anxiety risk reached 15.8%, especially obvious in the 18 - 24 age group. [2]

Anxiety disorders not only cause great distress to patients but also bring a heavy load to their families and society. It is essential to conduct more in - depth research on the pathological mechanisms of anxiety disorders and identify accurate and effective therapeutic targets. Such research efforts will improve our understanding of the disease, offer theoretical backing for the development of new treatment methods, and effectively enhance the quality of life of those affected, thus bringing about significant clinical and social advantages.

This paper carries out multi - faceted research on anxiety disorders through an exhaustive literature review. It systematically analyzes their epidemiology, pathological mechanisms, treatment methods, and the latest theoretical breakthroughs. Additionally, it explores new viewpoints in anxiety disorder research through imaging studies of brain structure and function, as well as analyses centered on the neurotransmitters serotonin (5 - HT) and gamma - aminobutyric acid (GABA). Although medications targeting 5 - HT and GABA have been commonly used, they are still among the limited number of effective treatments for anxiety disorders. Recent research and findings related to these neurotransmitters offer great potential for the future creation of innovative anti - anxiety drugs.

2. Overview of Anxiety Disorders

Anxiety disorders are common mental health issues marked by excessive, ongoing, and unmanageable worry and tension, even when no real danger is present. As defined in the DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition), these disorders constitute a group of conditions characterized by heightened fear and anxiety, along with associated behavioral disruptions. Fear is an emotional reaction to an immediate or perceived threat, while anxiety involves apprehension about future dangers. Although fear and anxiety share similarities, they also have distinct traits. Fear usually triggers the "fight or flight" response of the autonomic nervous system, often accompanied by a sense of immediate peril and a desire to escape. On the other hand, anxiety is more associated with muscle tightness and increased vigilance in anticipation of potential threats, frequently leading to cautious or evasive actions. In certain instances, individuals may reduce the severity of fear or anxiety through extensive avoidance strategies.

Panic attacks are a prominent feature of anxiety disorders, representing a specific form of fear response. While panic attacks can occur in various mental health conditions and are not unique to anxiety disorders, different subtypes of anxiety disorders can be identified by the specific stimuli that provoke

fear, anxiety, or avoidance, as well as by the related thoughts and beliefs. Despite the frequent co-occurrence of multiple anxiety disorders, a thorough clinical assessment can distinguish between them by analyzing the nature of feared or avoided situations and the underlying cognitive factors. [3]

2.1. Common Symptoms of Anxiety Disorder [3]

(1) Challenges in maintaining concentration or making informed decisions

(2) Experiencing irritability, tension, or a sense of unease

(3) Nausea or abdominal discomfort

(4) Palpitations

(5) Sweating, trembling, or shaking

(6) Insomnia

(7) A perception of being in danger, experiencing panic, or anticipating an adverse event

2.2. Types of Anxiety Disorders (Typically Classified by Scenarios Such as Social Situations) [3]

Generalized anxiety disorder (excessive and persistent worry about daily activities or events)

Panic disorder (panic attacks and the fear of having more panic attacks)

Social anxiety disorder (extreme fear and worry about social situations that may cause humiliation, embarrassment or rejection);

Agoraphobia (an excessive fear, worry and avoidance of situations that may cause a person to panic or feel trapped, helpless or embarrassed);

Separation anxiety disorder (excessive fear or worry about being separated from someone with whom one has a deep emotional bond)

Specific phobia (an intense, irrational fear of a specific object or situation, leading to avoidance behavior and extreme distress)

Selective mutism (a condition where a person is persistently unable to speak in certain social situations, although they can speak freely in other circumstances, mainly affecting children)

3. The Mechanisms of Anxiety Disorders and Changes in Brain Structure

Although there remain numerous controversies regarding the precise pathogenic sites of anxiety disorders, in recent years, with the advancement of neuroimaging techniques and neuroendocrine research, the biological mechanisms underlying anxiety disorders have gradually come to light. Extensive studies have demonstrated that individuals with anxiety disorders exhibit structural and functional abnormalities in specific brain regions, along with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. Currently, it is widely accepted that the vicious cycle contributing to anxiety disorders unfolds as follows: anxiety-inducing stimuli → hypothalamus → hippocampus → hypothalamus → pituitary → adrenal → increased cortisol levels → physical discomfort → negative outcomes → further stimulation of anxiety-inducing stimuli, ultimately leading to the exacerbation of the condition. These findings provide crucial evidence for elucidating the pathophysiological mechanisms of anxiety disorders, developing novel diagnostic tools, and identifying potential therapeutic targets. The amygdala, as a critical brain region involved in fear processing and autonomic regulation, exhibits bilateral volume reduction in patients with anxiety disorders, including generalized anxiety disorder (GAD) and panic disorder (PD) . [4,5] This structural alteration may compromise its regulatory capacity over threatening information, leading to heightened sensitivity and imbalanced responses to negative stimuli, thereby serving as the neuroanatomical basis for persistent anxiety symptoms. The volume reduction of the amygdala may abnormally enhance its sensitivity to fear-inducing stimuli, resulting in localized metabolic hyperactivity. The parahippocampal gyrus, which is closely linked to amygdalar function, demonstrates decreased gray matter density in anxiety disorders. [6] This brain region plays a pivotal role in integrating episodic and emotional memory; its structural abnormalities may disrupt the processing of fear-related memories, prompting excessive rumination and pathological recall of negative events, thus reinforcing the vicious cycle of anxiety. Structural changes in the basal ganglia and parahippocampal gyrus respectively potentiate the fixation of negative emotions and behavioral avoidance through motivational processing and memory integration, ultimately contributing to the persistence and recurrence of anxiety symptoms. The prefrontal cortex, particularly the right medial orbitofrontal cortex (rMOFC), a key node for emotion-cognition integration, shows reduced volume in patients with anxiety disorders. The structural modifications in this area may impair the cognitive appraisal of emotional stimuli, leading to diminished inhibitory control of the prefrontal cortex over excessive amygdalar activation, thereby disrupting the "prefrontal-limbic system" regulatory circuit. The atrophy and functional inhibition of the prefrontal cortex jointly weaken its regulatory capacity over the amygdala, causing bidirectional dysregulation in the "limbic system-prefrontal" circuit. [7] The reduction in gray matter volume within the basal ganglia (especially on the right side) is significantly specific to anxiety disorders. As a core component of the striatum-globus pallidus circuit, its structural changes may affect the motivational processing of threatening stimuli, intensifying the tendency toward avoidance behaviors in states of anxiety and forming pathological fixation at the behavioral level. [8] Patients with PD exhibit reduced metabolism in the left inferior parietal lobe, and the functional decline in this region may be associated with somatic symptoms (e.g., palpitations and suffocation) during panic attacks. Concurrently, glucose uptake in core brain regions of the fear circuit, such as the amygdala, hippocampus, and thalamus, is markedly increased. This metabolic enhancement is state-dependent, returning to normal levels following effective treatment, indicating reversible functional alterations rather than permanent damage. [9] Cerebral hemodynamic changes: The overall reduction in bilateral cerebral blood flow (CBF) is positively correlated with symptom severity, indicating a general trend of diminished whole-brain functional activity. Single-photon emission computed tomography (SPECT) reveals that under conditions of globally reduced activity in both frontal lobes, compensatory activation occurs in the medial and superior regions of the right frontal lobe. This phenomenon may represent an adaptive mechanism by which the brain enhances cognitive control and attempts to suppress excessive limbic system activation. However, the imbalance in this compensatory mechanism could potentially serve as the

neural substrate for recurrent panic attacks. Brain imaging studies of anxiety disorders demonstrate highly specific structural remodeling and dynamic functional abnormalities, with their interaction forming the neurobiological basis for disease onset and progression. [10-12]

4. The Evolution of Theories on the Causes of Anxiety Disorders

In 1965, Joseph Schildkraut published a landmark paper in the American Journal of Psychiatry that first proposed the possibility of depression being associated with insufficient norepinephrine (NE) function within the central nervous system. [13]Schildkraut hypothesized that norepinephrine was a neurotransmitter implicated in depressive disorders. Specifically, he suggested that reduced levels of norepinephrine in certain brain circuits could trigger depressive episodes, whereas elevated levels might induce manic states. In 1967, clinicians Alec Coppen and Joseph Schildkraut introduced the "permissive hypothesis," which posited that decreased serotonin levels could also lead to diminished norepinephrine activity. However, serotonin levels could be modulated to indirectly enhance norepinephrine function. Alec Coppen further expanded on the monoamine theory through tryptophan depletion experiments, proposing that reduced serotonin (5-HT) levels constituted a core pathological mechanism underlying depression. His research demonstrated that depressed patients exhibited significantly lower concentrations of 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of serotonin, in their cerebrospinal fluid compared to healthy controls. Furthermore, supplementation with tryptophan, a precursor of serotonin, alleviated symptoms in some patients. These findings were subsequently integrated with Schildkraut's earlier norepinephrine hypothesis to form the "monoamine imbalance theory," or the chemical imbalance theory. This theory gained traction in academic circles, particularly after the introduction of fluoxetine (Prozac), the first selective serotonin reuptake inhibitor (SSRI), in 1988. Fluoxetine's efficacy in increasing serotonin concentrations in the body contributed to its rapid adoption and reinforced the validity of the chemical imbalance theory. By the early 21st century, surveys indicated that over 80% of the general public believed depression resulted from "chemical imbalances in the brain." Despite its widespread acceptance, this theory encountered significant skepticism during the early 21st century. Most psychiatrists now acknowledge that depression and anxiety are not merely attributable to deficiencies in specific neurotransmitters but require more rigorous scientific validation to elucidate their underlying causes. In 2022, a systematic review published in Nature Molecular Psychiatry presented a significant challenge to the serotonin hypothesis. This study conducted a comprehensive and rigorous analysis of 17 brain imaging and cerebrospinal fluid studies involving over a thousand participants, concluding that there is insufficient empirical evidence to support the notion that depression is associated with reduced serotonin concentration or activity. The majority of studies found no clear association between serotonin levels and depression, while high-quality genetic studies excluded any meaningful link between serotonin system-related genotypes and depressive disorders. Some studies suggesting an association may have been confounded by the effects of antidepressant medications, and the results of tryptophan depletion studies remain inconclusive. The serotonin hypothesis lacks robust empirical support and has shaped public perceptions of depression as well as treatment decisions. However, this finding does not negate the role of the serotonin system but rather underscores its complexity beyond the simplistic concept of "concentration imbalance." [14] Numerous studies have demonstrated that currently available serotonin-based drugs, such as SSRIs and SNRIs, exhibit considerable efficacy. Moreover, multiple double-blind placebo-controlled trials have shown statistically significant differences in outcomes. [15] anxiety disorders and depression are distinct clinical entities, yet they share overlapping pharmacological treatments, particularly serotonin-based drugs. Nevertheless, notable differences exist in medication efficacy. For instance, benzodiazepines demonstrate greater effectiveness for anxiety disorder patients compared to those with depression. [16] Since the inception of research in 1965, spanning over six decades, the primary targets of antidepressant drugs have remained serotonin (5-HT) and GABA.

5. Treatment Targets and Drug Mechanisms for Anxiety Disorders

5.1. Serotonin Types and Anabolic Metabolism

Serotonin, a monoamine neurotransmitter, holds a pivotal role in both the central nervous system (CNS) and peripheral tissues. Its biosynthesis within the CNS mainly takes place in neurons located in the raphe nucleus of the brainstem. The process starts with the essential amino acid tryptophan, which needs to cross the blood-brain barrier to gain access to the CNS. Inside neurons, tryptophan undergoes enzymatic conversion to 5-hydroxytryptophan (5-HTP) through the action of tryptophan hydroxylase 2 (TPH2), followed by decarboxylation via L-aromatic amino acid decarboxylase (AADC) to generate serotonin (5-HT). The newly synthesized 5-HT is then transported into vesicles at axon terminals by vesicular monoamine transporter 2 (VMAT2). When an action potential occurs, 5-HT is released into the synaptic cleft, where it binds to 5-HT receptors on both pre- and postsynaptic membranes to exert its physiological effects. Subsequently, serotonin transporter (SERT) mediates the reuptake of 5-HT from the synaptic cleft for recycling. Additionally, 5-HT can be metabolized by monoamine oxidase-A (MAO-A) and aldehyde dehydrogenase, resulting in the formation of 5-hydroxyindoleacetic acid (5-HIAA).

The 5-HT receptor family consists of over 14 different subtypes, grouped into seven main classes: 5-HT_1 (1A, 1B, 1D, 1E, 1F), 5-HT_2 (2A, 2B, 2C), 5-HT_3, 5-HT_4, 5-HT_5 (5A, 5B), 5-HT_6, and 5-HT_7. Each subtype shows distinct distribution patterns and functions in the CNS and peripheral tissues. Subtypes potentially related to anxiety include members of 5-HT_1 (A, B, D), 5-HT_2 (A, B, C), 5-HT_4, 5-HT_6, and 5-HT_7 classes. Specifically, 5-HT_1 receptor subtypes such as 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} are involved in mood regulation, anxiety modulation, and pain perception. The 5-HT2 subtypes (2A, 2B, 2C) are primarily associated with psychiatric conditions like schizophrenia, depression, and anxiety disorders. Uniquely, the 5-HT_3 receptor acts as an ion channel-type receptor, mainly regulating nausea, vomiting, and intestinal motility. The 5-HT_4 receptor, expressed in both the CNS and gastrointestinal tract, contributes to cognitive functions and the regulation of gastrointestinal motility. The functions of 5-HT5 receptors remain partially understood but may be involved in sleep and circadian rhythm regulation in

the brain. The 5-HT_6 receptor, predominantly found in the CNS, is linked to learning and memory processes. The 5-HT7 receptor, one of the more recently identified subtypes, participates in mood regulation, sleep patterns, and body temperature control. Given the diversity and complexity of these receptor subtypes, the 5-HT system serves as a key therapeutic target for multiple disorders, including depression, anxiety, schizophrenia, pain syndromes, and gastrointestinal diseases. Further investigations into 5-HT receptors hold potential for developing more targeted and effective therapeutic agents, ultimately improving patient outcomes and quality of life. [17][19]

5.2. Frontier Research on 5-HT under Cryo-EM

5-hydroxytryptamine (5-HT) mediates pleiotropic effects through 12 G protein-coupled receptor (GPCR) subtypes and is regulated by various ligands and lipids, including phospholipids and cholesterol. Phosphatidylinositol-4-phosphate (PtdIns4P), as a critical phospholipid, functions as a mediator for GPCR-stimulated diacylglycerol generation. However, the precise mechanism by which PtdIns4P regulates GPCRs remains elusive due to the paucity of structural data. 5-HT receptors, such as 5-$HT_{1A/1D/1E}$, exhibit high basal activity and are coupled with Gi/Go proteins. Corresponding drugs, including aripiprazole and triptans, are utilized in the treatment of central nervous system disorders. Peiyu's research elucidated five cryo-electron microscopy (cryo-EM) structures (three 5-HT_{1A}, one 5-HT_{1D}, and one 5-HT_{1E} complexes), demonstrating that PtdIns4P is the predominant phospholipid at the interface between 5-HT1A and G proteins. [20]The 5-HT_{5A} receptor transmits signals via Gi/o proteins, playing a role in neural regulation and serving as a potential therapeutic target for anxiety and depression. The cryo-EM structure of the complex formed by 5-carboxamidotryptamine (5-CT) and the Gi protein (Gi alpha subunit), resolved at 3.1 Å, identified the receptor residue at position 6.55 as the determinant of 5-CT selectivity for Gi/o-coupled 5-HT receptors. The 5-HT_3 receptor, a pentameric ligand-gated ion channel (pLGIC), mediates ion permeability through conformational changes induced by extracellular ligand binding and serves as a therapeutic target for conditions such as vomiting, irritable bowel syndrome, and depression. This study determined the structures of four mouse 5-HT_3 receptors using cryo-EM, capturing conformations bound to tropisetron (inhibitory state, pore closed), serotonin (open state and two intermediate states), with resolutions ranging from 3.2 to 4.5 Å. [21]Tropisetron stabilizes the inhibitory conformation, whereas serotonin binding induces displacement of the transmembrane helix M2 and pore opening. Positive allosteric modulators enhance the stability of the open state. [22] [23]

5.3. Physiological Functions

In the central nervous system, serotonin is fundamental to regulating numerous physiological functions. It is deeply engaged in mood management, cognitive processes, anxiety regulation, learning, memory formation, reward evaluation, and sleep cycles. Specifically, in cognitive and memory functions, serotonin, through the diverse actions of 5-HT receptor subtypes, aids in the development of both associative and non-associative memories. When it comes to mood and reward mechanisms, serotonin works in tandem with dopamine to impact behaviors related to rewards, motivation, and the reinforcement effects of psychostimulants. The raphe nucleus stands out for its role in self-stimulation activation. Moreover, serotonin is crucial for pain modulation, controlling nociceptive input through the descending inhibitory system. Various 5-HT receptor agonists and antagonists have different impacts on pain regulation. Additionally, serotonin contributes to the regulation of circadian rhythms and interacts with the body's internal biological clock system. [23]

5.4. Selective Serotonin Reuptake Inhibitors (SSRI) and Their Mechanism of Action

Fluoxetine, sertraline, and paroxetine are typical examples of SSRIs. These medications boost extracellular serotonin levels by impeding the reuptake of serotonin into presynaptic neurons. Different SSRIs vary in their selectivity for other monoamine transporters. Pure SSRIs have a strong affinity for the serotonin transporter (SERT) and show only minimal interaction with norepinephrine and dopamine transporters.

The core action mechanism of SSRIs lies in inhibiting SERT on the presynaptic neuronal membrane. In normal physiological states, once serotonin (5-HT) is released into the synaptic cleft by presynaptic neurons, SERT quickly reabsorbs it, halting neurotransmission. SSRIs, however, selectively block this reuptake, causing an accumulation of 5-HT in the synaptic cleft. This prolongs the time 5-HT binds to postsynaptic receptors, enhancing its effects. The increased activation of postsynaptic receptors promotes better communication between neurons, which helps regulate mood and relieve anxiety and depressive symptoms.

Notably, although SSRIs rapidly increase synaptic 5-HT levels, their antidepressant and anxiolytic effects usually take several weeks to become apparent. This delay is related to subsequent adaptive changes in the brain, such as adjustments in receptor sensitivity and remodeling of neural networks. Thanks to their good safety and tolerability, SSRIs are commonly considered first-line treatments for many psychiatric conditions, including major depressive disorder, generalized anxiety disorder, and obsessive-compulsive disorder. [24][25]

5.5. Serotonin-Norepinephrine Reuptake Inhibitors (SNRI) and Their Mechanism of Action

Venlafaxine and duloxetine, as SNRIs (serotonin-norepinephrine reuptake inhibitors), have shown effectiveness in reducing anxiety symptoms and are commonly prescribed for individuals with co-occurring depressive disorders. These medications regulate depressive states by acting on neurotransmitters—chemical messengers that enable communication between nerve cells. Analogous to other antidepressant agents, SNRIs exert their therapeutic impacts through inducing neurochemical alterations in the brain, thereby enhancing signal transmission within neural circuits responsible for mood regulation.

The antidepressant, anxiolytic, and analgesic properties of SNRIs primarily stem from a dual-mode mechanism: selective inhibition of two presynaptic transporter proteins—the serotonin transporter (SERT) and norepinephrine transporter (NET). Under normal physiological conditions, serotonin (5-HT) and norepinephrine (NE) released into the synaptic cleft are quickly reabsorbed by these transporters, terminating their signaling role. By blocking SERT and NET, SNRIs impede this reuptake process, causing accumulation of

5-HT and NE in the synaptic space and prolonging their interaction with postsynaptic receptors. This enhancement of neurotransmission contributes to improved mood regulation and influences processes related to attention, energy levels, and pain perception. As a result, SNRIs are used in treating various psychiatric conditions, including major depressive disorder, generalized anxiety disorder, and obsessive-compulsive disorder, while also providing relief for neuropathic pain and fibromyalgia syndrome.

Notably, although SNRIs rapidly increase synaptic neurotransmitter concentrations, their clinical benefits usually emerge after several weeks. This temporal lag is thought to involve long-term adaptive mechanisms in the brain, such as receptor desensitization, neural plasticity, and remodeling of neuronal networks. In essence, by concurrently inhibiting reuptake of serotonin and norepinephrine, SNRIs enhance mood-related neurotransmission and modulate energy levels and pain processing, providing a strong pharmacological basis for managing diverse mental and physical health conditions. [24,25]

5.6. A Concise Overview of GABA

Gamma-aminobutyric acid (GABA) serves as the primary inhibitory neurotransmitter in the central nervous system, with its regulatory processes being essential for maintaining the balance between neuronal excitability and cognitive function. GABA receptors (GABARs) are broadly divided into two main classes: ligand-gated ion channel GABAA receptors and G protein-coupled GABAB receptors. Among these, the GABAA receptor is a pentameric protein complex composed of five subunits that form a central chloride ion channel. Its functional diversity stems from variations in subunit composition, which dictates the receptor's localization at synaptic sites (mainly on the postsynaptic membrane) versus extrasynaptic regions (spread across the neuronal membrane surface). This structural variability endows the receptor with distinct physiological functions and pharmacological characteristics.

The GABAA receptor subunit family includes multiple types, such as α1-6, β1-3, γ1-3, δ, ε, θ, and ρ1-3. These subunits assemble in a non-random manner, with the most common configuration consisting of two α subunits, two β subunits, and one γ subunit—for example, the α1β2γ2 subtype. This specific subunit arrangement creates two key ligand-binding domains: (1) a site located at the α-β subunit interface, which binds endogenous GABA and mediates rapid inhibitory synaptic transmission through chloride ion influx; and (2) a region formed by the α subunit (α1, 2, 3, or 5 subtypes) and the γ2 subunit, serving as the primary target for clinical sedative-anxiolytic drugs like benzodiazepines. These drugs enhance the opening frequency of GABA-gated chloride channels, thereby exerting central inhibitory effects. [19]

5.7. Synthesis and Metabolism of GABA

GABA is primarily synthesized via two major pathways. The first pathway involves the decarboxylation of glutamate, a reaction catalyzed by glutamate decarboxylase (GAD65 and GAD67), which produces GABA. Specifically, GAD67 exhibits widespread expression in neuronal cell bodies and throughout neurons, whereas GAD67 is predominantly localized at axon terminals. The second pathway entails the degradation of putrescine, mediated by monoamine oxidase B (MAO-B) or diamine oxidase (DAO). MAO-B is predominantly expressed in astrocytes and plays a critical role in regulating GABA tone within the cerebellum and striatum. Conversely, DAO is expressed in dopaminergic neurons and thalamic astrocytes, although its precise function in neurons remains debated. There are three primary mechanisms for GABA release: 1) Vesicular exocytosis: This process is predominantly mediated by inhibitory neurons through synaptic vesicles and serves as the principal mechanism for rapid post-synaptic inhibition. It may also contribute to the regulation of tonic inhibition. 2) Reverse transport by transporters: GABA is released via the reverse transport activity of GABA transporters (GATs); however, the physiological significance of this mechanism remains controversial. 3) Channel-mediated release: Channel proteins such as BEST1 and LRRC8A can directly facilitate GABA release. Notably, BEST1 participates in the modulation of tonic inhibition across multiple brain regions, while the role of LRRC8A is increasingly being elucidated. The clearance of GABA relies on an efficient uptake and metabolic system. First, transporter reuptake occurs via GAT1 and GAT3, which are highly expressed in the brain. Their differential distribution across various brain regions enables region-specific regulation of GABA uptake, thereby directly influencing local GABA tone. Second, enzymatic degradation transforms GABA into succinic acid through the sequential actions of GABA transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH). Inhibition of these enzymes significantly elevates GABA concentrations in the synaptic cleft, thereby enhancing inhibitory signal transmission. The subunit heterogeneity of GABAA receptors and the dynamic equilibrium of GABA collectively form the molecular foundation for inhibitory regulation in the brain. Elucidating the precise regulatory mechanisms provides pivotal targets for the development of therapeutic agents for anxiety, epilepsy, and other neurological disorders. [[26][27]

5.8. Some Frontier Research on GABA

The dual regulatory role of GABAergic neurons in the central nucleus of the amygdala: Acute restraint stress (ARS) has been shown to induce anxiety-like behavior in mice, characterized by prolonged grooming duration and increased respiratory frequency (RF). Mechanistically, silencing GABAergic neurons within the central amygdala (CeA) significantly alleviates anxiety-related behaviors, as well as the associated increases in grooming time and RF following ARS exposure. Further investigations have revealed that in behaviorally active mice, activation of CeA GABAergic neurons via the CeA-paraventricular thalamic nucleus (PVT) circuit induces anxiety-like behavior, prolongs grooming time, and elevates RF. Conversely, in behaviorally inactive or anesthetized mice, these neurons solely modulate RF through the CeA-lateral parabrachial nucleus (LPBN) circuit without inducing anxiety. These findings suggest that CeA GABAergic neurons regulate anxiety-related behavior (via the CeA-PVT circuit) and respiratory patterns (via the CeA-LPBN circuit) through two distinct and independent pathways. [28]

The association between reactive astrocytes and GABA tone dysregulation: Under pathological conditions, reactive astrocytes upregulate monoamine oxidase B (MAO-B), thereby enhancing the synthesis of γ-aminobutyric acid (GABA). This leads to an increase in extracellular GABA tone and intensifies tonic inhibition of neurons. This

mechanism plays a critical role in the pathophysiological processes of various brain disorders, including Alzheimer's disease, Parkinson's disease, stroke, depression, and obesity. Emerging research underscores the therapeutic potential of reversible MAO-B inhibitors (such as KDS2010) in targeting and mitigating abnormally elevated GABA tone. Additionally, elevated GABA tone and markers associated with reactive astrocytes (e.g., MAO-B activity) may serve as valuable biomarkers for disease diagnosis and monitoring.[29]

Bidirectional regulation of obesity-anxiety comorbidity by MC4R neurons in the dorsal bed nucleus of the stria terminalis: MC4R neurons located in the dorsal bed nucleus of the stria terminalis (dBNST) play a critical role in modulating the comorbidity of obesity and anxiety/depression through the integration of GABAergic and serotonergic signaling pathways. Chronic exposure to a high-fat diet (HFD) induces desensitization of AgRP neurons in the hypothalamus, leading to reduced GABA release onto dBNST MC4R neurons via $GABA_aR$-α5 receptors, while simultaneously activating 5-HT_3R, thereby causing excessive excitation of MC4R neurons. This hyperexcitation exacerbates anxiety and depressive behaviors and promotes feeding behavior. Genetic enhancement of $GABA_aR$-α5 signaling or inhibition of 5-HT_3R within MC4R neurons can effectively ameliorate behavioral abnormalities and reduce body weight. These findings provide novel insights into therapeutic strategies for comorbid conditions, suggesting that targeting the dual signaling pathways ($GABA_aR$-α5/5-HT_3R) of dBNST-MC4R neurons may serve as an effective approach for addressing both metabolic and psychological disorders. Furthermore, these studies elucidate the pivotal roles of neural circuits, glial cell functions, and receptor-mediated signals in brain diseases, paving the way for the development of targeted diagnostic markers and innovative treatment strategies, such as circuit-specific interventions, reversible enzyme inhibitors, and combination therapies. [30]

5.9. Benzodiazepines and Their Mechanism of Action

Benzodiazepines, including alprazolam, diazepam, lorazepam, clonazepam, and others, exhibit rapid onset of action, typically alleviating acute anxiety or panic attacks within 30 minutes to one hour. Owing to their pronounced sedative properties, these drugs effectively mitigate anxiety symptoms. However, prolonged or excessive use may result in dependence, tolerance, and adverse effects on cognition and memory. Consequently, benzodiazepines are generally prescribed for short-term use only.

The pharmacological effects of benzodiazepines are primarily mediated through modulation of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) in the central nervous system. These drugs do not directly activate GABA_A receptors but function as positive allosteric modulators. Specifically, they bind to the benzodiazepine binding site on GABA_A receptors, located between the α and γ subunits, thereby enhancing the affinity of GABA for its receptor. Upon GABA binding to its receptor, benzodiazepines increase the frequency of chloride ion channel openings, facilitating a substantial influx of chloride ions into neurons. This process induces hyperpolarization of the cell membrane and reduces neuronal excitability, clinically manifesting as anti-anxiety, sedative, anticonvulsant, muscle relaxant, and hypnotic effects.

Furthermore, the specific combinations of GABA_A receptor subunits influence certain clinical effects of benzodiazepines. For example, receptors containing the α1 subunit predominantly mediate sedative and hypnotic effects, whereas those with α2 and α3 subunits are more closely associated with anti-anxiety effects. It is crucial to emphasize that the efficacy of benzodiazepines relies on the presence of GABA; these drugs do not activate GABA receptors independently but significantly potentiate the inhibitory actions of GABA when it is present, thereby modulating neuronal activity. [24,25]

6. Conclusion

Anxiety disorders, as a globally prevalent mental health condition, have exhibited a persistent increase in prevalence rates, posing significant challenges to public health. The underlying pathological mechanisms involve structural remodeling of brain regions, dysregulation of neural circuits, and dysfunction of neurotransmitter systems, reflecting a highly intricate biological basis. From a neuroanatomical perspective, structural and functional abnormalities in the amygdala, parahippocampal gyrus, prefrontal cortex, and basal ganglia constitute the primary pathological foundation of anxiety disorders. Specifically, reduced amygdala volume contributes to an imbalance in threat signal processing, decreased gray matter density in the parahippocampal gyrus disrupts emotional memory integration, and weakened regulation of the prefrontal cortex over the limbic system leads to dysregulation of the "prefrontal-limbic" circuit, all of which exacerbate the persistence and intensity of anxiety symptoms. Functional imaging further reveals dynamic metabolic and hemodynamic abnormalities in specific brain regions. Among neurotransmitter systems, 5-hydroxytryptamine (5-HT) and γ-aminobutyric acid (GABA) serve as core regulatory targets. Although the serotonin hypothesis has faced challenges due to limitations in the "concentration imbalance" theory, the diverse roles of its receptor subtypes (e.g., 5-HT_1A and 5-HT_2A) in emotion regulation continue to inform drug development. The GABA system exerts anti-anxiety effects through inhibitory neurotransmission. Benzodiazepines target $GABA_aR$α2/α3 subunits for rapid alleviation of acute symptoms, while dual regulation mechanisms of GABAergic neurons in the amygdala on anxiety behavior and respiratory patterns offer novel avenues for circuit-specific interventions. Despite advances in understanding pathological mechanisms and therapeutic targets, current research faces several challenges: the heterogeneous pathogenesis of anxiety disorders remains incompletely understood, existing pharmacological treatments exhibit delayed onset, side effects, and drug resistance, and early precise diagnosis and personalized treatment strategies still require robust biomarkers. Future research should prioritize the following directions: first, integrating cutting-edge technologies such as single-cell sequencing and cryo-electron microscopy to elucidate brain region-specific functions of 5-HT/GABA receptor subtypes and interactions among multiple neurotransmitter systems; second, employing optogenetics and neural circuit tracing techniques to uncover dynamic regulatory mechanisms of core circuits like the "prefrontal-amygdala" and the neural underpinnings of comorbidities (e.g., depression and addiction); third, combining epigenetic and gut microbiota research to delineate the long-term impact of environment-gene interactions on anxiety susceptibility; and fourth, developing receptor subtype-selective drugs and advanced

neuroregulatory technologies (e.g., closed-loop deep brain stimulation) to facilitate the transition from symptom-based intervention to mechanism-based repair in precision medicine. In summary, research on anxiety disorders must transcend single-target approaches, construct a multidimensional integrated model encompassing "brain structure - neurotransmitter - behavioral phenotype," leverage interdisciplinary technical advancements and clinical big data, and ultimately achieve in-depth mechanistic insights, objective diagnostic criteria, and precise treatment plans, offering hope for millions of patients worldwide.

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