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Neuroinflammation in CNS disorders and their comorbidities: possible pharmacological control

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SUMMARY

Abstract

Neuroinflammation is increasingly considered as a central player in the development and progression of several CNS disorders, including both neuropsychiatric and neurodegenerative diseases. Chronic activation of microglia and astrocytes within the brain triggers the release of pro-inflammatory cytokines, chemokines, and reactive oxygen species, leading to synaptic dysfunction, imbalance in monoamine circuits, and neuronal damage. These processes are closely linked to the exacerbation of mood disorders, such as anxiety and depression, and contribute to long-term cognitive decline observed in neurodegenerative diseases, like Alzheimer's and Parkinson's. The modulation of neuroinflammatory pathways, as a novel pharmacological strategy, could be crucial in mitigating the harmful effects deriving from chronic inflammation, with the aim of improving neurological and psychiatric outcomes in affected patients. This thesis investigates these issues through different approaches. First, a high-fat diet (HFD)-induced obesity model in mice is used to examine how metabolic dysregulation contributes to neuroinflammation and anxiety-like behaviors. The study evaluates (1) the effect of Bisphenol A (BPA), a pro-inflammatory environmental pollutant, in worsening these outcomes, and (2) the therapeutic potential of 2-Pentadecyl-2-Oxazoline (PEA-OXA), a compound known for its anti-inflammatory properties, in alleviating both neuroinflammation and anxiety-related symptoms. Finally, a neonatal exposure of lipopolysaccharide (LPS) in rats is used to simulate early-life immune challenges, assessing the long-term neuropsychiatric consequences of early neuroinflammatory insults. This model recapitulates the development of Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS) and the role of early

inflammation in predisposing individuals to psychiatric disorders later in life. Together, these studies seek to provide novel insights into the mechanisms by which neuroinflammation contributes to CNS disorders.

Introduction

Neuroinflammation, a hallmark of several central nervous system (CNS) disorders, is increasingly recognized as a pivotal factor in the pathophysiology of both neurodegenerative and neuropsychiatric conditions [1]. This process involves the activation of immune cells within the CNS, primarily microglia and astrocytes, in response to various stimuli, including infection, trauma, toxins, and metabolic disturbances [2]. While the neuroinflammatory response is initially aimed at protecting and restoring homeostasis, chronic or dysregulated neuroinflammation can become deleterious, leading to neuronal damage and contributing to disease progression [3]. Neuroinflammation is characterized by the production and release of pro-inflammatory cytokines, chemokines, and reactive oxygen species (ROS), which exacerbate neuronal stress and dysfunction [4]. Microglial activation, in particular, is a central driver of this process. Upon activation, microglia shift from a homeostatic state to a reactive one, marked by the secretion of inflammatory mediators and enhanced phagocytic activity [2]. Although this response is essential for removing pathogens and debris during acute phases, persistent microglial activation can become harmful, leading to synaptic dysfunction, neuronal loss, and disrupted plasticity, fundamental processes linked to cognitive decline and psychiatric symptoms [3]. One of the major challenges in understanding neuroinflammation is in its dual role, while it is essential for brain defense and repair, it can also act as a pathogenic mechanism if uncontrolled [1]. This delicate balance between protective and harmful inflammation underscores the complexity of immune responses within the CNS. It is now clear that even subtle dysregulation of the brain's immune environment can

result in profound alterations in neural circuits, potentially triggering or exacerbating neurological and psychiatric diseases [4]. Neurodegenerative disorders like Alzheimer's, Parkinson's, and multiple sclerosis are all associated with chronic neuroinflammation, where continuous activation of glial cells contributes to progressive neuronal injury and functional deficits [2]. Similarly, neuroinflammation is increasingly implicated in mood disorders, such as depression and anxiety, where inflammatory cytokines can modulate neurotransmitter systems, neurogenesis, and synaptic function, leading to altered emotional and cognitive states [5]. Recent advances in neuroimmunology have also underscored the importance of peripheral immune system interactions with the CNS [1]. The blood-brain barrier (BBB), once considered an impermeable shield, is now understood to be a dynamic interface, facilitating selective communication between the brain and the peripheral immune system [3]. Under conditions of systemic inflammation, such as during infection or metabolic imbalance, pro-inflammatory cytokines can disrupt BBB integrity, allowing peripheral immune cells and mediators to infiltrate the CNS [5]. This interplay between peripheral and central immune responses amplifies neuroinflammation, illustrating the systemic nature of many CNS disorders [2]. Both genetic and environmental factors play significant roles in modulating susceptibility to neuroinflammation and its downstream effects [4]. Genetic predispositions may alter the expression or activity of key inflammatory regulators, while environmental factors such as stress, diet, and exposure to toxins, can further trigger or exacerbate neuroinflammatory responses [5]. The interaction between intrinsic genetic vulnerabilities and extrinsic environmental insults creates a complex landscape of risk and

resilience, influencing the onset, progression, and severity of CNS disorders [3]. Despite significant advances, gaps remain in understanding the precise mechanisms by which acute neuroinflammatory responses transition to chronic states and how they contribute to distinct neuropsychiatric and neurodegenerative conditions [2]. These knowledge gaps offer both challenges and opportunities for the development of therapeutic interventions. Targeting key regulators of neuroimmune responses presents a promising strategy for modulating neuroinflammation, aiming to restore CNS homeostasis without compromising the brain's innate defense mechanisms [4].

In this context, the role of neuroinflammation in CNS disorders and their behavioral comorbidities will be explored through three experimental models. First, the impact of a high-fat diet (HFD) on obesity-induced neuroinflammation and anxiety in mice will be examined, with a focus on two compunds: Bisphenol A (BPA), a molecule known to exacerbate anxiety [6], and 2-Pentadecyl-2-oxazoline (PEA-OXA), a compound with anti-inflammatory properties that may improve anxiety-like behaviors [7]. Second, the effects of early-life immune challenges on long-term neuropsychiatric outcomes will be investigated using a neonatal lipopolysaccharide (LPS) administration model in rats, aiming to understand the development of Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS) and the role of early neuroinflammatory insults in shaping CNS vulnerability to psychiatric disorders later in life [8].

Through these experimental approaches, this thesis seeks to uncover novel insights into the mechanisms of neuroinflammation in CNS disorders, exploring potential pharmacological strategies and risk

factors that modulate inflammatory pathways, thereby improving knowledge on behavioral outcomes and their mechanisms.

1. Obesity-induced anxiety

Obesity is a complex and multifactorial condition that significantly impacts both physical and mental health [9]. The interplay between obesity and anxiety is driven by various biological, psychological and environmental factors, including alterations in hormonal regulation, inflammation, and changes in the gut-brain axis [9]. Obesity-induced anxiety is characterized not only by an elevated risk of anxiety disorders but also by a bidirectional relationship, where anxiety may contribute to behaviors that exacerbate obesity, such as emotional eating and reduced physical activity [10,11]. From a biological perspective, adipose tissue in obesity secretes pro-inflammatory cytokines, which contribute to chronic systemic low-grade inflammation, also called metainflammation, and neuroinflammation [12]. The activation of the inflammatory pathways has been implicated in the onset and development of anxiety by disrupting the regulation of neurotransmitters like serotonin and dopamine [13]. Furthermore, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, a common feature in both obesity and anxiety, exacerbates stress responses, leading to heightened anxiety [9]. In addition to neurobiological mechanisms, obesity is often associated with psychological stressors such as body dissatisfaction, societal stigma, and social isolation. These factors act as chronic stressors, further aggravating anxiety symptoms [14].

The growing prevalence of both obesity and anxiety underscores the need to better understand their interrelationship. This chapter aims to explore the mechanisms by which obesity contributes to anxiety, focusing on inflammation, hormonal dysregulation, and the gut-brain axis alteration. Additionally, it will examine the implications of this

relationship for treatment and intervention strategies aimed at mitigating both obesity and anxiety in affected populations.

1.1 Anxiety: definition, epidemiology, diagnosis and risk factor.

Anxiety disorders are a heterogeneous group of psychiatric conditions characterized by excessive fear, worry, or apprehension, often in response to situations that are perceived as threatening or overwhelming, but which do not pose an immediate danger [15]. The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) categorizes various forms of anxiety disorders, including generalized anxiety disorder (GAD), panic disorder, social anxiety disorder (SAD), specific phobias, separation anxiety disorder, and agoraphobia, with each disorder presenting distinct diagnostic criteria and symptomatology [16]. Despite their differences, these disorders share a common underlying feature of persistent and uncontrollable anxiety, often leading to significant impairment in social, occupational, and daily functioning [17]. Anxiety disorders are also closely related to obsessive-compulsive disorder (OCD) and post-traumatic stress disorder (PTSD), which have been separated into distinct categories in the DSM-5 due to their unique clinical characteristics, though they share some overlapping features, such as hypervigilance and excessive worry [18].

Epidemiological data highlight the substantial global burden of anxiety disorders, which are among the most prevalent mental health disorders worldwide [19,20]. According to the Global Burden of Disease Study, anxiety disorders contribute significantly to years lived with disability (YLDs), particularly in high-income countries, but they also affect populations in low- and middle-income nations, where access to

mental health services is often limited [21]. The World Health Organization (WHO) estimates that approximately 264 million people globally suffer from some forms of anxiety disorder, with a lifetime prevalence of 15-20% (2017). Notably, the prevalence of anxiety disorders is higher in women than in men, with women being almost twice as likely to develop these conditions [22]. This gender disparity may be attributed to hormonal fluctuations, particularly those related to the menstrual cycle, pregnancy, and menopause, as well as psychosocial factors, including the higher prevalence of gender-based violence and caregiving responsibilities among women [23].

Anxiety disorders often have an early onset, with a median age of onset around 11 years, making them one of the earliest psychiatric disorders to manifest [24]. The chronicity and relapsing nature of anxiety disorders can significantly affect an individual's quality of life, leading to academic difficulties in childhood, impaired career development in adulthood, and strained interpersonal relationships [25]. Comorbidity with other psychiatric conditions, such as major depressive disorder (MDD), substance use disorders, and eating disorders, is highly prevalent. Kessler et al. [24] found that approximately 50% of individuals diagnosed with an anxiety disorder also suffer from depression, which complicates treatment and worsens prognosis. This frequent comorbidity underscores the need for comprehensive diagnostic assessments that consider the possibility of multiple co-occurring disorders.

The diagnosis of anxiety disorders is primarily clinical and relies on standardized diagnostic tools [26]. Structured clinical interviews, such as the Structured Clinical Interview for DSM-5 (SCID-5) and the Mini International Neuropsychiatric Interview (MINI), are widely used for

assessing the presence of anxiety disorders based on DSM-5 criteria [16]. These interviews are complemented by self-report scales, such as the Generalized Anxiety Disorder-7 (GAD-7) and the Hamilton Anxiety Rating Scale (HAM-A), which help quantify symptom severity and monitor treatment outcomes [27]. Although these tools are highly effective in clinical settings, there remains a challenge in distinguishing anxiety disorders from closely related conditions such as somatic symptom disorders, especially when anxiety manifests as physical symptoms like palpitations, dizziness, or gastrointestinal disturbances [28].

The etiology of anxiety disorders is complex and multifactorial, involving genetic, neurobiological, psychological, and environmental components [29]. Heritability estimates suggest that genetic factors account for approximately 30-50% of the variance in the development of anxiety disorders [30]. Variants in genes related to neurotransmitter systems, particularly those involving serotonin (5-HT), gamma-aminobutyric acid (GABA), and glutamate, have been implicated in the pathophysiology of anxiety [18]. Dysregulation of the HPA axis, a central stress response system, has also been consistently associated with anxiety disorders. Chronic hyperactivation of the HPA axis leads to sustained elevations in cortisol levels, which can adversely affect brain regions such as the amygdala and prefrontal cortex (PFC), both of which play critical roles in the regulation of fear and emotion [6]. Neuroimaging studies have demonstrated that individuals with anxiety disorders often exhibit hyperactivity in the amygdala in response to threat-related stimuli, as well as impaired functioning in the PFC and hippocampus, which are responsible for modulating emotional responses [31].

Environmental factors, such as early-life stress, childhood trauma, and chronic exposure to adversity, are significant contributors to the onset of anxiety disorders [32,33]. Numerous studies have demonstrated that individuals who experience neglect, abuse, or other forms of trauma during childhood are at an increased risk for developing anxiety disorders later in life [34]. Socioeconomic factors, including poverty, unemployment, and social isolation, further exacerbate the risk of anxiety, particularly in populations with limited access to healthcare and social support systems [21]. Additionally, cultural and social factors, such as stigma surrounding mental health, can delay the seeking of care and perpetuate the cycle of untreated anxiety [35]. Treatment for anxiety disorders generally involves a combination of pharmacotherapy and psychotherapy. Selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) are considered first-line pharmacological treatments due to their efficacy in regulating neurotransmitter activity and their relatively favorable side-effect profiles [36]. Benzodiazepines, though effective in the short-term management of acute anxiety symptoms, are typically reserved for short-term use due to the risk of dependence and withdrawal [37]. Cognitive-behavioral therapy (CBT) is the most well-established psychotherapeutic intervention, with strong evidence supporting its efficacy across multiple anxiety disorders [38]. CBT targets maladaptive thought patterns and behaviors, helping individuals reframe their anxiety-provoking thoughts and develop healthier coping mechanisms [38]. In recent years, novel therapeutic approaches, such as mindfulness-based therapies and acceptance and commitment therapy (ACT), have gained attention for their potential

to address anxiety symptoms through non-judgmental awareness and acceptance of distressing thoughts [39].

Despite the availability of effective treatments, a substantial proportion of individuals with anxiety disorders do not seek help [40]. Stigma, lack of access to mental health care, and financial barriers are common reasons for untreated anxiety, particularly in low-income settings [40]. Moreover, among those who do receive treatment, response rates can vary, and a significant proportion of patients experience partial or non-response to first-line treatments [41]. This highlights the need for continued research into personalized treatment approaches, including the identification of biomarkers that can predict treatment response and the development of new pharmacological and psychotherapeutic modalities that can target treatment-resistant forms of anxiety [29].

Summarizing, anxiety disorders are highly prevalent, multifaceted mental health conditions with a significant global burden. Their etiology is shaped by a complex interplay of genetic, neurobiological, and environmental factors, and their course is often complicated by early onset, comorbidity, and chronicity. While current treatments are effective for many individuals, challenges remain in improving access to care, reducing stigma, and enhancing treatment outcomes. Future research should focus on understanding better the biological mechanisms underlying anxiety, improving diagnostic accuracy, and developing innovative, personalized treatment strategies to reduce the global burden of anxiety disorders.

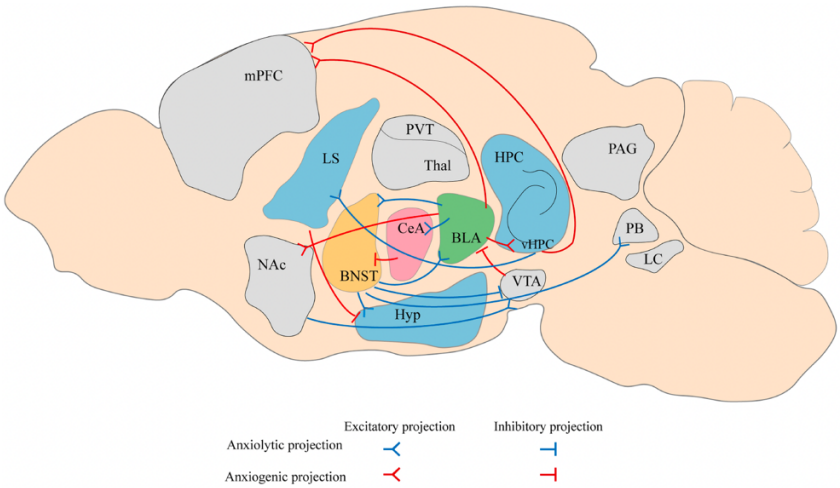

Fig. 1. Anxiety neural circuit. Anxiety states involve dysregulated neural communication across multiple interconnected brain regions. Key areas implicated in these processes include the basolateral amygdala (BLA), bed nucleus of the stria terminalis (BNST), central amygdala (CeA), hippocampus (HPC), hypothalamus (Hyp), locus coeruleus (LC), lateral septum (LS), medial prefrontal cortex (mPFC), nucleus accumbens (NAc), periaqueductal gray (PAG), parabrachial nucleus (PB), paraventricular thalamus (PVT), thalamus (Thal), ventral hippocampus (vHPC), and ventral tegmental area (VTA)(Zhao et al., 2023).

1.2 Pathogenic mechanisms of anxiety

Anxiety disorders, a broad category of mental health conditions characterized by excessive worry, fear, and apprehension, have complex and multifactorial pathogenic mechanisms [15]. Understanding the biological underpinnings of these disorders involves unraveling the interactions between genetic predispositions, neurobiological dysfunctions, environmental stressors, and systemic factors such as inflammation [29]. Among these factors, the role of neuroinflammation in the development and perpetuation of anxiety has gained increasing attention in recent years [42]. Emerging evidence suggests that neuroinflammatory processes may contribute to the onset and maintenance of anxiety symptoms by disrupting neural circuits involved in emotion regulation, fear processing, and stress responses

[6]. While genetic and neurochemical imbalances have long been established as central to the pathogenesis of anxiety disorders [29], the interplay between chronic inflammation and altered neural function provides a more comprehensive model of how these disorders develop [43].

At the genetic level, heritability studies have demonstrated that anxiety disorders are moderately heritable, with genetic factors accounting for 30-50% of the variance in susceptibility [30]. Several genes involved in the regulation of neurotransmitter systems, particularly those related to serotonin (5-HT), dopamine, and gamma-aminobutyric acid (GABA), have been implicated in anxiety disorders [44]. Variants in the *SLC6A4* gene, which encodes the serotonin transporter (5-HTT), have been associated with altered 5-HT reuptake and have been consistently linked to both GAD and panic disorder [44]. Dysfunction in serotonergic signaling is believed to impair the regulation of mood and stress responses, predisposing individuals to heightened anxiety [45]. Similarly, dysregulation of the GABAergic system, the primary inhibitory neurotransmitter in the central nervous system, has been linked to impaired neuronal inhibition, resulting in increased neuronal excitability and hypervigilance, key features of anxiety disorders [46].

Neuroplasticity, the brain's ability to adapt and reorganize in response to stress and environmental stimuli, is also disrupted in anxiety disorders [47]. Chronic stress, a known risk factor for anxiety, triggers long-lasting changes in neural circuits, particularly those involving the amygdala, hippocampus, and PFC [6]. The amygdala, responsible for processing fear and emotional stimuli, is hyperactive in individuals with anxiety disorders, while the PFC, which exerts top-down control to regulate emotional responses, shows hypoactivity [48]. This

imbalance between hyperactivity in the amygdala and reduced regulatory control from the PFC is thought to be a hallmark of the pathophysiology of anxiety [49]. Importantly, these neuroplastic changes are influenced by both genetic predispositions and environmental stressors, including early-life adversity, trauma, and chronic psychosocial stress, which can lead to structural and functional changes in these brain regions[50].

In recent years, a growing number of research has focused on the role of neuroinflammation in anxiety disorders [51]. Neuroinflammation refers to the activation of the brain's immune cells, particularly microglia and astrocytes, in response to infection, injury, or stress [52]. This inflammatory response leads to the release of pro-inflammatory cytokines, including interleukin (IL)-1β, tumor necrosis factor (TNF)α, and interleukin (IL)-6, which can alter neuronal functioning and impair synaptic plasticity [43]. Chronic low-grade neuroinflammation has been linked to various psychiatric disorders, including depression and anxiety [53]. In anxiety disorders, the persistent activation of the immune response in the central nervous system (CNS) can lead to disruptions in neural circuits that regulate fear and stress responses [29]. One of the key pathways linking neuroinflammation and anxiety involves the HPA axis, the central stress response system [54]. Chronic stress leads to the sustained activation of the HPA axis, resulting in the continuous release of cortisol [54]. While cortisol exerts anti-inflammatory effects in the periphery, prolonged HPA axis activation can disrupt glucocorticoid receptor function in the brain, reducing the capacity to regulate inflammation [55]. This impaired glucocorticoid signaling allows pro-inflammatory cytokines to persist in the brain, further exacerbating neuroinflammation [54]. Importantly, elevated

levels of IL-6, TNFα, and other cytokines have been observed in individuals with anxiety disorders, particularly in those who experience comorbid depression or chronic stress [53]. These cytokines can directly influence neuronal circuits by reducing the availability of key neurotransmitters, including 5-HT and GABA, through mechanisms such as oxidative stress and disruption of monoamine metabolism [56].

The involvement of microglia, the resident immune cells of the CNS, is particularly relevant in the context of neuroinflammation and anxiety [57]. Microglia serve as the brain's primary line of defense against infection and injury, and under normal conditions, they maintain homeostasis by clearing debris and regulating synaptic pruning [3]. However, in response to chronic stress or peripheral immune activation, microglia become chronically activated, releasing inflammatory mediators that can induce neurotoxicity [57]. In anxiety, chronic microglial activation has been shown to contribute to synaptic dysfunction and altered neuroplasticity, particularly in brain regions implicated in emotion regulation, such as the hippocampus and amygdala [14]. Studies have demonstrated that stress-induced activation of microglia leads to increased production of IL-1β, which in turn activates the nuclear factor (NF)-κB signaling pathway, a key mediator of the inflammatory response [58]. NF-κB activation has been implicated in anxiety-like behaviors in animal models, suggesting a direct link between neuroinflammation and the behavioral manifestations of anxiety [58].

Astrocytes, another type of glial cell, also play a critical role in neuroinflammation and anxiety [59]. Astrocytes regulate neurotransmitter levels in the synaptic cleft, maintain the BBB, and

provide metabolic support to neurons [60]. In the context of anxiety, astrocytes can become dysfunctional under conditions of chronic stress, leading to impaired glutamate uptake and subsequent excitotoxicity [59]. Glutamate is the primary excitatory neurotransmitter in the CNS, and its dysregulation has been implicated in the pathogenesis of anxiety disorders [61]. Overactivation of glutamate receptors, particularly N-methyl-D-aspartate (NMDA) receptors, can lead to increased neuronal excitability, contributing to the hyperactivity of fear circuits, particularly in the amygdala [62]. Inflammatory cytokines, particularly IL-1β and TNFα, have been shown to disrupt astrocytic glutamate transport, leading to increased synaptic glutamate levels and heightened anxiety-like behavior [63].

Peripheral inflammation can also influence anxiety through the "gut-brain axis" [64], a bidirectional communication network between the gut microbiota, the immune system, and the brain [65]. Emerging research suggests that alterations in the gut microbiome can promote systemic inflammation and neuroinflammation, contributing to the development of anxiety [66]. Dysbiosis, or an imbalance in gut microbiota composition, has been associated with increased intestinal permeability, called "leaky gut" [67], allowing bacterial endotoxins such as lipopolysaccharide (LPS) to enter the bloodstream and trigger an immune response [68]. LPS-induced inflammation has been shown to increase circulating levels of pro-inflammatory cytokines, which can cross the BBB and exacerbate neuroinflammation [69]. Preclinical studies have demonstrated that probiotic interventions that restore microbial homeostasis can reduce anxiety-like behaviors in animal models, highlighting the potential therapeutic implications of targeting the gut-brain axis in anxiety disorders [70].

In summary, the pathogenic mechanisms underlying anxiety disorders involve a complex interplay of genetic, neurochemical, and environmental factors, with neuroinflammation playing a particularly pivotal role in the onset and perpetuation of symptoms [51]. Chronic activation of immune cells, particularly microglia and astrocytes, leads to the release of pro-inflammatory cytokines that disrupt neurotransmitter systems and synaptic plasticity, impairing the function of neural circuits responsible for regulating fear, stress, and emotion [71]. Moreover, peripheral inflammation, whether induced by stress or gut dysbiosis, can further exacerbate neuroinflammatory processes, linking systemic immune dysfunction to central anxiety pathways. Given the growing evidence supporting the role of neuroinflammation in anxiety, therapeutic strategies aimed at modulating inflammatory pathways, such as anti-inflammatory agents, probiotics, or targeted interventions to regulate microglial activation, represent promising avenues for the treatment of anxiety disorders.

1.3 The crosstalk between anxiety and obesity

The interrelationship between obesity and anxiety disorders has become a critical focus of research in the field of mental health and obesity medicine [11]. Obesity, defined as having a body mass index (BMI) of 30 or greater, affects over 42% of adults in the United States, creating a significant public health challenge [72]. Concurrently, anxiety disorders represent the most prevalent mental health disorders in the U.S., affecting approximately 19.1% of adults annually [29]. The association between these two pathological conditions is complex and multifactorial, with emerging evidence highlighting

neuroinflammation as a pivotal mechanism linking obesity to anxiety [73].

Obesity is characterized by a state of chronic low-grade inflammation that can significantly affect neurobiological processes involved in mood regulation and emotional well-being [43]. Adipose tissue, particularly visceral fat, produces a variety of pro-inflammatory cytokines, including IL-6, TNFα, and CRP [43]. These cytokines not only promote systemic inflammation but also are capable to cross the BBB, leading to neuroinflammation in the CNS [60].

Neuroinflammation is known to disrupt the balance of neurotransmitters essential for mood regulation, particularly 5-HT and gamma-aminobutyric acid (GABA) [44]. Elevated levels of IL-6 and TNFα have been associated with dysregulation of serotonergic and GABAergic systems, which may exacerbate anxiety symptoms in individuals with obesity [56]. For instance, the modulation of 5-HT levels has been shown to directly influence anxiety-like behaviors in preclinical models, where inflammation-induced changes in 5-HT metabolism led to heightened anxiety responses [74].

The HPA axis, a critical component of the body's stress response system, is also impacted by the interplay between obesity and anxiety [75]. Chronic stress, often prevalent in individuals with obesity, activates the HPA axis, resulting in the excessive release of cortisol, a glucocorticoid hormone [6]. While cortisol initially serves to counteract inflammation, chronic exposure can lead to glucocorticoid receptor resistance, impairing the brain's ability to regulate inflammatory responses effectively. This dysregulation creates a feedback loop in which elevated cortisol levels promote further neuroinflammation, exacerbating anxiety symptoms [54].

Moreover, research has indicated that individuals with obesity often exhibit increased HPA axis reactivity to psychosocial stressors, which may further amplify anxiety symptoms [76].

The psychological dimensions of obesity and anxiety are equally important in understanding their interconnection [77]. Anxiety disorders often lead individuals to engage in maladaptive coping mechanisms, such as emotional eating, which can contribute to weight gain and perpetuate anxiety symptoms [78]. A study by Appelhans et al. [79] showed that individuals with higher levels of anxiety were more likely to consume high-calorie foods in response to stress, highlighting the role of emotional factors in the obesity-anxiety relationship. Additionally, the stigma associated with obesity can provoke feelings of social anxiety and low self-esteem [80], further entrenching individuals in a cycle of emotional distress and unhealthy eating behaviors [80].

Neuroimaging studies have provided insight into the structural and functional changes in the brain associated with both obesity and anxiety. Research by Alonso-Caraballo et al. [73] has demonstrated that obese individuals exhibit alterations in brain regions responsible for emotional regulation, including the anterior cingulate cortex and the amygdala. These changes may contribute to heightened anxiety vulnerability, as the amygdala plays a crucial role in processing fear and emotional stimuli [14]. Furthermore, preclinical studies have shown that high-fat diets can induce neuroinflammation and disrupt the integrity of neural circuits associated with anxiety regulation [74].

The role of microglia, the resident immune cells in the CNS, is particularly relevant in the context of neuroinflammation and anxiety [81]. Under normal conditions, microglia maintain homeostasis by

regulating synaptic pruning and clearing cellular debris [82]. However, in response to chronic stress or obesity-related inflammation, microglia can become activated and release inflammatory mediators that lead to neurotoxicity [57]. Chronic microglial activation has been shown to disrupt synaptic plasticity in brain regions implicated in emotional regulation, such as the hippocampus and amygdala [83].

Research has indicated that stress-induced activation of microglia results in increased production of pro-inflammatory cytokines [42], such as IL-1β, which activates the NF-κB signaling pathway, a key mediator of the inflammatory response [84]. This pathway has been linked to anxiety-like behaviors in animal models, suggesting a direct connection between neuroinflammation and the behavioral manifestations of anxiety [58]. Moreover, elevated levels of IL-6 and TNFα have been observed in individuals with anxiety disorders, particularly in those experiencing comorbid depression or chronic stress [85]. Another crucial aspect of the obesity-anxiety relationship involves the gut-brain axis [64], which represents a communication network between the gut microbiota, the immune system, and the brain. Dysbiosis, namely an imbalance in gut microbiota composition, can lead to increased intestinal permeability, allowing bacterial endotoxins, such as LPS, to enter the bloodstream and trigger systemic inflammation [66]. Preclinical studies have demonstrated that LPS-induced inflammation can increase circulating levels of pro-inflammatory cytokines, which can cross the BBB and exacerbate neuroinflammation [69]. Furthermore, interventions targeting gut microbiota through probiotics have shown promise in reducing anxiety-like behaviors in animal models [86,87]. A study by Bravo et al. [70] highlighted that probiotic treatment could ameliorate anxiety-like

symptoms and restore microbiota balance, suggesting that therapeutic approaches aimed at modulating the gut-brain axis may offer a novel strategy for managing anxiety in individuals with obesity.

The relationship between obesity and anxiety is intricate, underpinned by shared biological mechanisms, including neuroinflammation. Chronic activation of immune cells, particularly microglia and the secretion of pro-inflammatory cytokines, disrupts neurotransmitter systems and synaptic plasticity, impairing the function of neural circuits responsible for regulating fear, stress, and emotional responses. Additionally, the psychosocial aspects of obesity contribute to a cycle of anxiety and unhealthy coping mechanisms, further complicating the clinical picture. Given the compelling evidence supporting the role of neuroinflammation in the interplay between obesity and anxiety, future research should focus on elucidating the specific pathways involved in this relationship. Investigating the potential for anti-inflammatory interventions, such as dietary modifications, probiotics, or pharmacological agents targeting inflammation, may provide promising avenues for the treatment of both conditions. Integrating approaches that address both the psychological and biological dimensions of obesity and anxiety will be essential in developing comprehensive treatment strategies aimed at improving mental health outcomes in affected individuals.

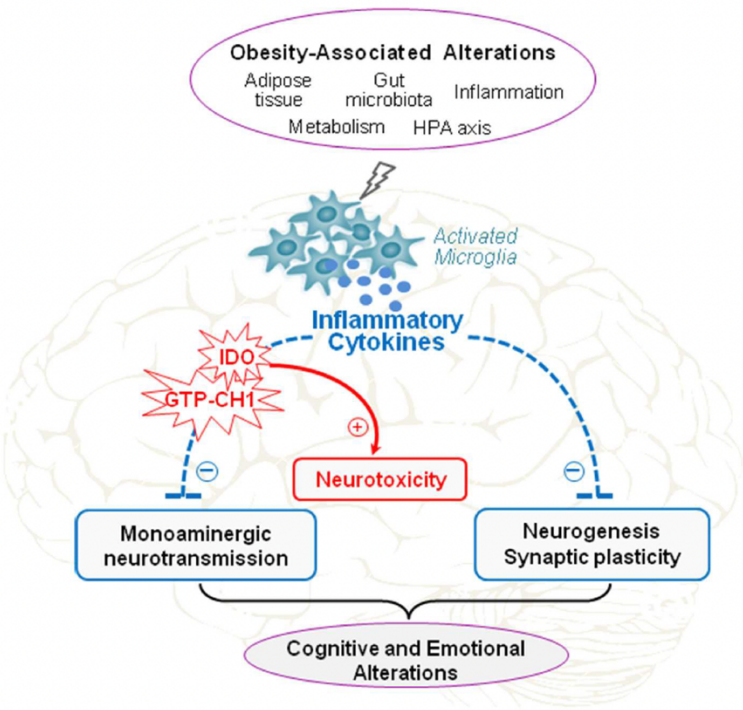

Fig. 2. Obesity and neuroinflammation. Obesity is characterized by metabolic dysregulations, including hyperinsulinemia, hyperleptinemia, and heightened activation of the hypothalamo-pituitary-adrenal (HPA) axis. Additionally, it is linked to low-grade systemic inflammation, stemming primarily from changes in adipose tissue and gut functionality. These obesity-related disruptions are well-established contributors to neuroinflammatory processes, which play a central role in the emergence of behavioral changes associated with obesity (Castanon er al., 2015).

1.4 Anxiety and Bisphenol A

Bisphenol A (BPA), a well-known endocrine-metabolic disruptor, represent a significant public health concern due to its pervasive presence in everyday products and its ability to interfere with hormonal and metabolic homeostasis [88]. BPA, commonly found in polycarbonate plastics and epoxy resins used in food containers, water bottles, and thermal paper receipts, has been shown to leach into food and water, leading to widespread human exposure [89]. Studies have

shown that even low-level exposure to BPA can disrupt endocrine functions due to its structural similarity to endogenous hormones like estrogen, allowing it to bind to estrogen receptors (ERα and ERβ), thereby altering physiological regulatory mechanisms [90,91]. This interference with the estrogenic pathway has broad metabolic implications, as estrogen is involved in regulating glucose homeostasis, lipid metabolism, and stress responses [92]. Importantly, such metabolic disruptions have been increasingly linked to neuropsychiatric conditions, including anxiety disorders [93].

BPA's interference with the HPA axis, a key regulator of stress responses, plays a central role in anxiety modulation [6]. Studies have found that BPA exposure alters cortisol secretion patterns, leading to heightened stress sensitivity and the promotion of anxiety-like behaviors [94]. Rodent studies on animal models have consistently demonstrated that prenatal and postnatal exposure to BPA results in abnormal corticosterone levels, the rodent analog of cortisol, which is strongly associated with anxiety-like behavior [93]. Such dysregulation is also linked to disruptions in neurotransmitter systems, particularly 5-HT and dopamine (DA), which are essential for mood and emotional regulation [91,95]. BPA's ability to interfere with these systems may contribute significantly to the onset or exacerbation of anxiety-related symptoms.

Moreover, BPA's influence extends beyond the endocrine system to include metabolic disruptions that exacerbate its role in anxiety disorders [6]. BPA exposure has been linked to insulin resistance, increased adipogenesis, and obesity, all of which are recognized as comorbid conditions with anxiety and depression [89,96]. These metabolic dysfunctions result from BPA-induced oxidative stress and

inflammation, which are known to worsen neuroinflammatory processes [97]. For instance, BPA exposure elevates reactive oxygen species (ROS) levels, leading to mitochondrial dysfunction and neuronal damage, particularly in the hippocampus and amygdala [96]. Concurrently, BPA's ability to trigger chronic inflammation is linked to elevated levels of pro-inflammatory cytokines, such as IL-6 and TNF-α, which have been associated with both heightened anxiety and depressive symptoms [93,98].

The clinical implications of these findings are profound, as they suggest that BPA acts not only as an endocrine disruptor but also as a metabolic disruptor that heightens anxiety risk via multiple biological pathways [92]. Studies have highlighted vulnerable populations, such as pregnant women, children, and individuals with high occupational exposure to BPA, who may be particularly susceptible to its adverse effects [99]. As the associations among BPA exposure, metabolic dysfunction, and anxiety become increasingly evident, more research is necessary to elucidate the precise molecular mechanisms through which BPA operates. This will be critical in guiding the development of targeted therapeutic interventions to mitigate the neuropsychiatric consequences of BPA exposure. Furthermore, public health policies must aim to reduce BPA exposure by regulating its use in consumer products and raising awareness of its potential risks [92]. Ultimately, BPA’s role as a disruptor of both endocrine and metabolic pathways places it at the crossroads of key processes essential for mental health, particularly in relation to anxiety disorders. Understanding these interactions will be vital for addressing the broader public health impacts of environmental toxicants on mental well-being [96].

1.5 2-Pentadecyl-2-oxazoline

2-Pentadecyl-2-oxazoline (PEA-OXA), a synthetic derivative of palmitoylethanolamide (PEA), has received increasing attention due to its ability to modulate neuroinflammation and pain [100]. Preclinical studies highlight its therapeutic potential, particularly in the treatment of neuroinflammatory conditions, chronic pain, and mood disorders [100,101]. The molecular mechanisms underlying PEA-OXA's effects have been linked to its interaction with multiple receptor systems: the molecule can inhibit the N-acylethanolamine acid amide hydrolase (NAAA), the enzyme responsible for the degradation of endogenous PEA [7]. However, recent studies have shown that the molecule interacts with histamine H3 and adrenergic α_2 autoreceptors. In silico docking studies demonstrated that PEA-OXA binds to H_3 receptors, inhibiting histamine release, and similarly modulates adrenergic α_2 receptors, which play a crucial role in the central nervous system's response to inflammation and neurotransmission [101,102]. This receptor modulation has been corroborated in experimental models of inflammation and neuropathic pain, where PEA-OXA effectively suppressed the release of pro-inflammatory cytokines like TNFα, IL-1β, and IL-6 [100]. PEA-OXA's anti-inflammatory properties are especially evident in neurodegenerative models, where chronic inflammation is a key pathological feature [102]. For instance, in a Parkinson's disease model, PEA-OXA reduced neuroinflammation and dopaminergic neuron loss, an effect attributed to its activation of the Nrf2 pathway, which regulates cellular responses to oxidative stress [103]. This mechanism was also supported by findings in models of multiple sclerosis and Alzheimer's disease, where PEA-OXA administration led to a decrease in oxidative stress markers and

improved neurological outcomes [102]. In addition to modulating oxidative pathways, PEA-OXA was shown to downregulate NF-κB, further reducing the progression of neuroinflammation [104].

The compound's effects extend beyond anti-inflammatory activity. In models of neuropathic pain, PEA-OXA demonstrated potent analgesic effects, alleviating mechanical allodynia and thermal hyperalgesia [105]. These outcomes are believed to be mediated through its interactions with the endocannabinoid system and peroxisome proliferator-activated receptor (PPAR)-α, both of which play pivotal roles in pain perception and inflammatory modulation [106]. Furthermore, the interaction of PEA-OXA with histamine H3 receptors appears to contribute to improved cognitive function and emotional regulation, which are often impaired in conditions of chronic pain and neurodegeneration [107]. In animal models of depression and anxiety, PEA-OXA significantly improved behavior, reducing depression-like and anxiety-like symptoms through modulation of neurotransmitter systems linked to both mood and pain [101]. This broad neuroprotective activity places PEA-OXA as a compelling candidate for managing psychiatric symptoms associated with neurodegenerative diseases and chronic pain conditions. Moreover, PEA-OXA has demonstrated efficacy in reducing acute inflammation in non-neurological models, such as carrageenan-induced inflammation [106], where it was shown to inhibit local edema formation and neutrophil infiltration. These findings suggest that PEA-OXA could also be beneficial in treating peripheral inflammatory conditions [108]. Such data highlights the versatility of PEA-OXA in modulating both central and peripheral inflammatory processes, making it an attractive candidate for further pharmacological development.

In summary, PEA-OXA represents a novel therapeutic tool, particularly for disorders involving inflammation and neuroinflammation. Its ability to interact with multiple receptors, such as histamine H3, adrenergic a_2, PPAR-α and NAAA, allows it to exert pleiotropic effects across various physiological systems. The results from preclinical studies suggest that PEA-OXA is significantly promising for the treatment of neurodegenerative diseases, chronic pain, and mood disorders, though further research is necessary to elucidate its full potential in clinical settings.

2. Pediatric Acute Onset Neuropsychiatric Syndrome (PANS)

Pediatric acute-onset neuropsychiatric syndrome (PANS) is a complex disorder characterized by the sudden onset of neuropsychiatric symptoms, most notably severe obsessive-compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD), autism spectrum disorder, schizophrenia and other behavioral abnormalities, often in response to an immune or environmental trigger [109]. Early-life immune activation, particularly following perinatal exposure to various pathogens, has been strongly linked to PANS, with both bacterial and viral agents implicated in its etiology [110,111]. While streptococcal infections are often highlighted in PANS' closely related condition, pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS), a variety of other pathogens, such as *Mycoplasma pneumoniae*, *Escherichia Coli*, and even the SARS-CoV-2 virus, have been associated with the onset of PANS symptoms [112].

Here, the diagnostic criteria, clinical presentation, and pathophysiological mechanisms of PANS will be explored, highlighting the role of immune system dysregulation, neuroinflammation, and molecular mimicry. Additionally, the potential implications of LPS-induced models will be examined in the understanding of the neuroinflammatory processes in PANS, with an emphasis on how systemic inflammation may exacerbate or trigger the symptoms seen in this syndrome.

2.1 PANS: Diagnostic criteria and clinical presentation

Early-life immune activation induced by perinatal exposure to different pathogens has been associated with the development of many neuropsychiatric disorders named PANS [109,113]. Indeed, PANS has been primarily associated with streptococcal infections (PANDAS); however, other different pathogens (bacteria and viruses, including *Escherichia Coli*, *Bartonella spp*, *Mycoplasma pneumoniae*, Epstein–Barr virus, Influenza virus, Varicella-zoster virus, SARS-CoV-2) are linked to the onset of neuropsychiatric disturbance [114]. However, in some cases, no clear infectious trigger is identified, underscoring the heterogeneity of this syndrome. The SARS-CoV-2 outbreak, and the following spread of COVID-19, also brought pre-existing neuropsychiatric symptoms to the surface, inducing behavioral abnormalities [115]. The acute onset of neuropsychiatric symptoms in PANS is striking, often described as occurring "overnight" or within 24 to 48 hours, with parents reporting that their previously healthy child has undergone a profound and sudden behavioral change [112]. Clinically, the presentation of PANS is highly variable, although the rapid onset of a severe OCD is a hallmark. Children may also present other neuropsychiatric symptoms, such as extreme mood swings, severe anxiety or panic attacks. Emotional instability is common, with patients demonstrating sudden outbursts of rage or irritability, as well as depressive symptoms [116]. Additionally, motor abnormalities such as tics, choreiform movements, or motor skill deterioration are often observed [117]. Cognitive regression is another significant feature, with children experiencing difficulties in memory, concentration, and academic performance [118]. In addition to the neuropsychiatric symptoms, sleep disturbances, including insomnia or excessive

sleepiness, are commonly observed, and many children exhibit urinary frequency or urgency, suggestive of autonomic nervous system involvement [112]. These symptoms, along with the neuropsychiatric presentation, tend to follow an episodic course, with periods of symptom exacerbation followed by partial or complete remission [8]. Exacerbations are often triggered by environmental stressors, including infections or psychological stress, highlighting the immune and neuroinflammatory components of the syndrome [119]. Research suggests that PANS is associated with an abnormal immune response, with some studies indicating the presence of neuroinflammation, as well as the production of autoantibodies that may target the basal ganglia, a brain region involved in motor control and behavior [120]. Due to the complexity of PANS, early diagnosis and intervention are crucial to preventing long-term neuropsychiatric consequences. Treatment is typically multimodal and tailored to the individual patient. If an infectious trigger is suspected, antibiotics may be administered, and anti-inflammatory treatments, such as corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), or intravenous immunoglobulin (IVIG), are often used to mitigate the immune response [121]. Psychiatric treatment is also essential for symptom management, particularly for OCD and anxiety, with cognitive-behavioral therapy (CBT) being a key intervention [122,123]. Selective serotonin reuptake inhibitors (SSRIs) are sometimes used, but children with PANS are often highly sensitive to these therapeutics [124]. PANS is a multifaceted syndrome characterized by the acute onset of severe neuropsychiatric symptoms, often in response to an immune or environmental trigger. The disorder's broad clinical spectrum, coupled with its fluctuating course, underscores the need for

a multidisciplinary treatment approach. Early recognition and timely intervention are critical to improving outcomes, as untreated PANS can result in chronic and debilitating neuropsychiatric conditions [109].

PANDAS Criteria 1998	PANS Criteria 2012
1. Presence of diagnosis of OCD and/ or tic disorder	1. Abrupt, dramatic onset of OCD or severely restricted food intake
2. Pediatric onset	2. Concurrent presence of additional neuropsychiatric symptoms (with similarly severe and acute-onset) from at least two of the following seven categories
3. Episodic course	A. Anxiety
4. Temporal association with GAS infection	B. Emotional lability and/or depression
5. Association with neurologic abnormalities	C. Irritability, aggression, and/or severely oppositional behaviors D. Behavioral (developmental) regression E. Deterioration in school performance F. Sensory or motor difficulties G. Somatic signs or symptoms, including sleep disturbances, enuresis, or urinary frequency
	3. Symptoms are not better explained by a known neurologic or medical disorder, such as SC, systemic lupus erythematosus, Tourette syndrome, or others

Table 1. Diagnostical criteria for PANDAS and PANS (Vreeland et al., 2023).

2.2 Pathophysiology and Immune System in PANS

The pathophysiology of PANS is strongly associated with immune dysregulation, particularly involving molecular mimicry, autoantibody production, and inflammation within the CNS [125]. In this context, multiple immune mechanisms are implicated in driving the neuropsychiatric symptoms characteristic of PANS. Molecular mimicry is a central hypothesis in the immune-mediated mechanisms underlying PANS [126]. This process occurs when immune responses generated against microbial antigens wrongly target self-antigens in the brain due to structural similarities between microbial proteins and neuronal proteins [127]. In PANS, autoantibodies produced against the pathogens cross-react with neuronal targets, especially within the basal ganglia, which is involved in regulating motor control, behavior, and emotions [128]. Key autoantibodies implicated in PANS include those targeting dopamine D1 and D2 receptors, lysoganglioside GM1, and tubulin, all of which have been identified in both PANS and related

conditions like PANDAS [128]. These autoantibodies interfere with dopamine signaling and alter synaptic transmission in the basal ganglia, contributing to the motor and behavioral symptoms observed in patients [129]. Dopamine dysregulation, particularly in the striatum, is thought to exacerbate neuropsychiatric symptoms like obsessive-compulsive behaviors and tics [128]. In addition to autoantibodies, immune dysregulation in PANS involves excessive activation of the complement system [130]. In PANS, complement activation contributes to neuroinflammation and neuronal damage, further disrupting the neural circuits involved in behavior and cognition [119]. The role of pro-inflammatory cytokines is also critical in PANS pathophysiology [131]. Studies have shown elevated levels of cytokines such as IL-6 and IL-17 in patients with PANS [132]. These cytokines promote inflammatory responses and are involved in T-cell activation and differentiation. IL-17 has been implicated in mediating inflammation in autoimmune diseases by promoting the recruitment of neutrophils and amplifying the immune response [133]. In the context of PANS, the overproduction of these cytokines can exacerbate neuroinflammation, leading to disruptions in synaptic plasticity and neuronal signaling in brain regions like the basal ganglia and cortex [128,129]. The dysregulated immune response not only causes direct neuronal damage but also disrupts normal neurotransmitter balance particularly within the dopaminergic system, which is crucial for regulating mood and behavior [134]. The basal ganglia, a group of nuclei in the brain involved in motor and cognitive functions, are particularly vulnerable to immune-mediated attacks in PANS [111]. Neuroimaging studies have demonstrated changes in the structure and function of the basal ganglia in PANS patients, including increased activation in response to

inflammatory mediators and autoantibodies [128]. Damage to the basal ganglia can manifest as motor tics, emotional dysregulation, and cognitive impairments symptoms commonly seen in PANS [109].

In addition to structural damage, dysfunction in the basal ganglia results in altered neurotransmission [135], particularly involving the dopaminergic system [136]. Dopamine is a key neurotransmitter involved in reward processing, mood regulation, and motor control. Autoantibodies that target dopamine receptors (D1R and D2R) in the basal ganglia impair dopamine signaling, leading to the motor abnormalities, anxiety, and obsessive-compulsive behaviors that are hallmarks of PANS [129].

2.3 LPS-induced Behavioral Changes

LPS, an endotoxin found in the outer membrane of Gram-negative bacteria, is widely used in preclinical models to study the effects of immune activation on behavior [137]. LPS activates the innate immune system by binding to Toll-like receptor (TLR)4 on immune cells, triggering the release of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α [138]. These cytokines induce systemic inflammation and can influence the brain, causing behavioral changes that resemble symptoms of depression and anxiety [56]. Behavioral changes associated with LPS administration include "sickness behavior," which is characterized by lethargy, reduced exploration, social withdrawal, and anhedonia (inability to experience pleasure) [139]. This sickness behavior is considered an adaptive response to infection; however, in preclinical models, it also serves as a proxy for studying depressive-like states [140]. The release of cytokines, particularly IL-1β, IL-6, and TNF-α, is thought to play a critical role in mediating these behaviors.

These cytokines can signal the brain through several pathways, including crossing the BBB or activating peripheral nerves such as the vagus nerve, which transmits inflammatory signals to brain regions responsible for mood and behavior, such as the hypothalamus and prefrontal cortex [56,141]. One of the key molecular mechanisms underlying LPS-induced behavioral changes is the activation of microglia [142], the brain's resident immune cells. LPS stimulates microglial activation, leading to increased production of pro-inflammatory cytokines within the CNS [143]. This neuroinflammatory response disrupts neurotransmitter systems, particularly the serotonergic and dopaminergic pathways, which are critical for regulating mood and motivation [140]. Moreover, neuroinflammation caused by LPS has been shown to impair neurogenesis in the hippocampus, a brain region important for learning, memory, and emotional regulation [144]. Reduced neurogenesis is a prediction of many mood disorders, suggesting that inflammation-induced impairment of neuroplasticity is a key factor linking immune activation to behavioral alterations [52].

In addition to cytokine signaling, LPS-induced inflammation also alters the functioning of the HPA axis [145]. LPS increases the production of corticotropin-releasing hormone (CRH) and cortisol, leading to hyperactivation of the HPA axis. Recent studies have also explored the role of oxidative stress in LPS-induced behavioral changes. LPS triggers the production of ROS [146], which can cause damage to neurons and contribute to neuroinflammation [147]. Elevated oxidative stress is associated with reduced levels of brain-derived neurotrophic factor (BDNF), a protein essential for neuronal survival and synaptic plasticity. The downregulation of BDNF in response to

LPS may further exacerbate behavioral deficits by impairing neuroplasticity and cognitive function [52]. Overall, LPS-induced preclinical models have significantly advanced our understanding of how immune activation can lead to neuropsychiatric disorders. By simulating infection-induced inflammation, these models have provided insight into the mechanisms by which cytokines, oxidative stress, and HPA axis dysregulation contribute to behavioral changes. These findings have important implications for understanding the role of inflammation in neuropsychiatric disorders, and they suggest potential therapeutic strategies aimed at targeting inflammation and restoring neuroplasticity.

2.4 LPS and PANS

LPS has garnered attention for its potential role in triggering neuroinflammatory responses that could contribute to the pathogenesis of PANS. In the context of PANS, this systemic inflammatory response could amplify neuroinflammation, particularly in vulnerable brain regions like the basal ganglia, brain region widely implicated in [111]. The relationship between LPS-induced inflammation and neuropsychiatric disorders is well-documented in preclinical models [53,141]. LPS administration in animal studies consistently produces "sickness behaviors," such as social withdrawal, anhedonia, and lethargy, which mirror symptoms seen in psychiatric disorders, including those observed in PANS patients [13,56]. These behaviors are closely linked to the actions of pro-inflammatory cytokines, which disrupt normal neurotransmitter functioning, particularly in the dopaminergic and serotonergic systems [82]. Since dopaminergic dysregulation is a hallmark of basal ganglia dysfunction, it has been

proposed that similar mechanisms may be at play in PANS, where immune activation can lead to neuroinflammatory damage and behavioral abnormalities, including obsessive-compulsive behaviors and tics [117]. Furthermore, studies in animal models show that LPS-induced neuroinflammation is associated with the activation of microglia [142,148]. Microglial activation leads to the production of additional pro-inflammatory molecules within the CNS, exacerbating neuroinflammation and potentially triggering or worsening PANS symptoms [149]. This cascade of events can further impair BBB integrity, allowing even more peripheral cytokines and immune cells to enter the CNS, thus sustaining the inflammatory process [150]. Chronic or recurrent infections, which can involve LPS exposure, may lead to prolonged microglial activation and long-term changes in neuroplasticity, further implicating immune-mediated mechanisms in the progression of neuropsychiatric symptoms in PANS. Interestingly, LPS exposure is also linked to the production of autoantibodies, which may cross-react with neuronal proteins [49]. In PANS, autoantibodies targeting neuronal structures, such as dopamine receptors and tubulin, have been identified, suggesting a potential autoimmune component triggered by infections [128]. LPS could play a role in this by stimulating the immune system to produce such autoantibodies, further contributing to the neuropsychiatric manifestations of PANS [112]. The presence of these autoantibodies has been observed to interfere with neurotransmission, particularly in the basal ganglia, which is consistent with the clinical presentation of motor tics and obsessive-compulsive behaviors related to PANS [129].

3. AIMS

The aim of this thesis is to investigate the role of neuroinflammation in the development and progression of central nervous system (CNS) disorders and the related behavioral impairments, with a particular focus on anxiety and PANS. Specifically, we seek to explore the mechanisms by which environmental factors, such as nutritional habits (i.e. HFD intake) or bacterial infection (driven by LPS) affect neuroinflammatory processes, leading to alterations in mood, and long-term neurological outcomes. This thesis will address these aims evaluating

1. **the impact of a high-fat diet (HFD) on obesity-induced neuroinflammation and its relationship with anxiety-like behaviors in mice.**

   We aim to elucidate the neuroinflammatory pathways triggered by metabolic dysregulation caused by a HFD, focusing on the contribution of pro-inflammatory cytokine release. Additionally, we assess the role of neuroinflammation in the development of anxiety-like behaviors and examine the modulation of these pathways by Bisphenol A (BPA) and 2-Pentadecyl-2-oxazoline - oxazoline (PEA-OXA). Specifically, **BPA** was investigated for its pro-inflammatory and anxiety-exacerbating effects, hypothesizing its potential in amplifying the neuroinflammatory response, leading to worsened behavioral alterations. Moreover, **PEA-OXA**, known for its anti-inflammatory properties, was tested for its potential to mitigate both neuroinflammatory markers and anxiety-like behaviors, to address a possible therapeutic avenue.

2. **the effects of early-life exposure to lipopolysaccharide (LPS) on long-term neuroinflammatory and neuropsychiatric outcomes in rats.**

We modelled early-life immune challenge by administering LPS to neonatal rats and assessed the development of neuropsychiatric conditions resembling PANS. This experiment seeks to (1) characterize the neuroinflammatory response to neonatal LPS exposure, with particular focus on cytokines, neurotransmitters and ROS production in key brain regions, and (2) evaluate neuropsychiatric symptoms, including those related to OCD, anxiety, and schizophrenia, hypothesizing that early neuroinflammatory insults predispose individuals to psychiatric disorders later in life.

4. Materials and Methods

4.1 Ethical statements

Animal care and experimental procedures were carried out in compliance with international and national law and policies and approved by the local animal care office (Centro Servizi Veterinari, Università degli Studi di Napoli, Federico II) and the Italian Ministry of Health under protocol no. 371/2017-PR and no. 591/2020-PR. Animal studies complied with the Italian DL (No. 26 of 4 March 2014) of the Italian Ministry of Health and the EU Directive 2010/63/EU for animal experiments, ARRIVE guidelines, and the Basel declaration, including the 3Rs concept.

4.2 Diet-induced obesity experiments:

4.2.1 Diet-induced obesity

Male C57Bl/6J mice (Charles River Laboratories, Wilmington, MA, USA) at 6 weeks of age were kept in stainless steel cages at 22 ± 1 ◦C with a 12:12 h lights-dark cycle. The animals were divided into two groups: control group (STD) receiving chow diet (Mucedola Srl, Milan, Italy) and HFD (Research Diets Inc., NJ, USA) group feed with a high-fat diet. STD diet had 17% fat without sucrose while HFD had 45% energy derived from fat and 7% of sucrose. 12 weeks after HFD feeding, the animals underwent the treatments.

4.2.2 Treatments

BPA administration (50 μg/kg per os, ≥ 99% purity, Sigma-Aldrich, Milan, Italy) lasted 3 weeks. BPA was dissolved in 0,01% EtOH and corn oil to facilitate oral gavage. Until 2015, the scientific community has regarded this BPA dose as a tolerable daily exposure for humans.

The treatment with PEA-OXA (30 mg/kg/die per os; Epitech Group SpA) started from week 12 up to week 19. PEA-OXA was dissolved in 4% carboxymethylcellulose. All animals were subjected to behavioral tests accordingly with the protocol, as specified below. At the end of both experimental designs, animals were sacrificed by cervical dislocation following blood sampling.

4.3 LPS-induced PANS experiment

Male and female Wistar rats at post-natal day (PND) 3 were intraperitoneally injected with either a vehicle solution (0.9% saline; Saline group) or lipopolysaccharide (LPS, 1 mg/kg) (LPS group). After injection, the pups were returned to cages with their mothers until weaned on PND21, then transferred to new cages. Behavioral testing was conducted at PND42, PND43, and PND44, with rats undergoing open field, marble burying, and self-grooming tests, respectively. On PND45, the animals were sacrificed for the collection of brain tissue and serum samples.

A summary of the experimental design is provided in Fig 13A.

4.4 Behavioral tests

4.4.1 Open field test

The Open Field Test (OFT), along with the Elevated Plus Maze Test (EPMT), is among the most widely utilized assays for assessing anxiety-like behaviors in rodents. This test allows researchers to gather data on thigmotaxis, which refers to the tendency of mice to avoid open spaces perceived as threatening. In this procedure, mice were placed in an open-field arena and allowed to explore for a duration of 30 minutes per trial. The arena measured 50 cm in length, 50 cm in width, and 38

cm in height. Animals' movements were recorded using an infrared video camera and analyzed with EthoVision XT video tracking software (Noldus Information Technology, Wageningen, Netherlands). The following parameters were evaluated: (1) total distance travelled (m); (2) average velocity (m/s); (3) percentage of distance covered in the central area, calculated as [(distance travelled in the center / total distance travelled) × 100] or time spent in the same area; and (4) the number of entries into the central area.

4.4.2 Elevated Plus Maze test

The aversion of mice to open spaces and elevated areas is commonly assessed using the Elevated Plus Maze Test (EPMT). The maze (Ugo Basile apparatus, Gemonio, Italy) consists of four arms (5 cm in width and 35 cm in length): two opposite arms are enclosed by black walls (20 cm in height), while the other two arms remain open. The central platform where all four arms intersect serves as the access point to the maze. The test begins by placing each mouse individually in the center of the apparatus oriented towards an open arm and allowing them to explore for 5 minutes. The number of entries into the open and closed arms, as well as the time spent in each zone, were recorded and analyzed using EthoVision XT video tracking software (Noldus Information Technology, Wageningen, Netherlands). An arm entry is defined by the placement of the head into an arm. The percentage of time spent in the closed arms is regarded as a primary indicator of an 'anxiety-like' state. Additionally, the total distance travelled (m) and average speed (m/s) were monitored to assess the locomotor activity of the mice.

4.4.3 Marble Burying

Psychomotor agitation, characterized by stereotyped and hyperactive movements, was evaluated using the marble burying and self-grooming tests.

In the marble burying test, twenty ceramic marbles were arranged in a standard cage on a five-centimeter layer of woodchips. The marbles were evenly spaced in five rows of four. A rat was then introduced into the cage and allowed to explore freely for 30 minutes. Afterward, the rat was removed, and the number of marbles buried (defined as being at least 75% covered by woodchips) was recorded. This count served as the primary measure for assessing stereotyped behaviors.

4.4.4 Self-Grooming

The rats were placed alone in clean cages for five minutes. During this time, each investigator monitored a single cage, using a stopwatch to record the duration of self-grooming behavior. Self-grooming was defined as obsessive scratching of the face with the front paws, licking of the back of the body, or both.

4.5 Biochemical and molecular parameters

4.5.1 Bio-Plex assay

Serum levels of 23 inflammatory cytokine and immune mediators (G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1β, MCP-1, IL-18, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12 (p-70), IL-13, IL-17α, M-CSF, MIP-1α, MIP-3α, RANTES, TNF-α and VEGF) were measured with the Bio-Plex System and Luminex xMAP technology (Bio-Rad Laboratories, Inc., USA) using a high sensitivity kit (Bio-Techne; R&D Systems, Inc., USA). The color-coded beads are pre-coated with analyte-specific

capture antibodies which bind to the cytokine of interest. Then, detection antibodies specific to the analytes of interest are added. Finally, phycoerythrin-conjugated streptavidin is added, binding to the biotinylated detection antibodies in a final volume of 100 µL. Cytokine concentrations were calculated by interpolating the measured fluorescence intensities to a standard curve and correcting by the corresponding dilution factor used to reach the minimum volume for analysis. The final cytokines concentrations were assessed using the Bio-Plex Manager software.

4.5.2 RNA extraction and semi-quantitative Real-time PCR (RT-PCR)

Total RNA from samples was extracted using TRIzol Reagent (Bio-Rad Laboratories, Hercules, CA, USA) and isolated with the extraction kit's protocol for RNA (NucleoSpin®, MACHEREY-NAGEL GmbH & Co, Düren, Germany). cDNA was obtained using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) from 4 µg of total RNA. PCRs were performed with a Bio-Rad CFX96 Connect Real-time PCR System instrument and software (Bio-Rad Laboratories). The PCR was performed in the following described conditions: 15 min at 95°C, 40 cycles of two step PCR denaturation at 94°C for 15s and annealing extension at 55°C for 30s and extension at 72°C for 30s. Each sample contained 500 ng cDNA in 2X QuantiTect SYBRGreen PCR Master Mix and primers pairs to amplify *Tnf*, *Il1b*, *Nlrp3*, *Tlr4*, *Ccl2*, *Crh*, *Crhr1*, *Tpsb2*, *Cma1*, *Nfkb1*, *Ocln*, *Cldn5*, *Dcx1*, *Egr1*, *mKi67* in a final volume of 50 µl. Actin β (Qiagen, Hilden, Germany) was used as a housekeeping gene to normalize each studied mRNA, and data were analyzed according to the $2^{-\Delta\Delta C_T}$ method.

4.5.3 Western-Blot Analyses

Samples were homogenized in lysis buffer (20 mM Tris–HCl, pH 7,5, 10 mM NaF, 150 mM NaCl, 1% Nonidet P-40, 1 mM phenylmethylsulphonyl fluoride, 1 mM Na3VO4, leupeptin 10 μg/mL, and trypsin inhibitor 10 μg/mL). Total protein lysates were obtained as supernatant by centrifugation at 14,000 × g for 15 min at 4°C. Protein concentrations were estimated by the Bio-Rad protein assay using fetal bovine serum albumin (BSA) as standard. An equal amount of protein (40 μg) was subjected to SDS-PAGE and electro-transferred onto a nitrocellulose membrane using a Bio-Rad Transblot (Bio-Rad, Milan, Italy). Membranes were blocked at room temperature in milk buffer (1X PBS, 5% w/v non-fat dry milk) and incubated overnight at 4ºC with the following primary antibody: anti-TLR4 (1:200; Invitrogen, Whaltam MA, USA; 48-2300), anti-COX-2 (1:500; Elabscience, Houston, TX, USA), anti-BiP (1:1000; Cell Signaling Technology Inc., Danvers, MA), anti-p-eIF2α (1:1000; Cell Signaling Technology Inc., Danvers, MA, USA), anti-eIF2α (1:1000; Cell Signaling Technology Inc., Danvers, MA, USA), anti-PTP1B (1:500; Santa Cruz Biotechnology, Dallas, TX, USA), anti-p-GSK3β (1:1000; Cell Signaling Technology Inc., Danvers, MA, USA), anti-GSK3β (1:1000; Cell Signaling Technology Inc., Danvers, MA), anti-p-AKT (1:1000; Santa Cruz Biotechnology, Dallas, TX, USA), anti-AKT (1:1000; Santa Cruz Biotechnology, Dallas, TX, USA). Western Blot for anti-β-Actin (1:8000; Sigma-Aldrich St. Louis, MO, USA) was performed to ensure equal sample loading and data were expressed as relative normalized expression. The bans detection was performed by

ChemiDoc Imaging System (Bio-Rad Laboratories, Hercules, CA, USA).

4.6 Data and statistical analysis

4.6.1 Diet-induced Obesity experiments

Data are presented as mean ± SEM. Analysis of variance (ANOVA) for multiple comparisons followed by Bonferroni's post hoc test was performed to analyze all experiments using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). Bonferroni's post hoc test was run only when F was significant. The significant differences among groups were expressed at least at values of $p < 0.05$.

4.6.2 Pans induction by LPS Challenge experiment

All data shown are presented as mean value ± SEM. Intragender and intergender comparisons were made by two-way analysis of variance (ANOVA) followed by Bonferroni post hoc for multiple comparisons. Differences among groups were considered significant at values of $P < 0.05$. All Analyses were performed using GraphPad Prism 10 (GraphPad Software, San Diego, CA, USA).

5. Results

5.1 Effects of BPA on obese mice

5.1.1 BPA worsens anxiety-like behavior induced by HFD in OFT and EPMT

We assessed the behavioral patterns of mice in the OFT (Fig. 3A), focusing on the time taken to explore the exposed central areas, whose reduction is typically linked to anxiety-like behavior. When analyzing the distance covered, HFD mice exposed to BPA predominantly moved along the edges of the arena. Specifically, this group exhibited a decreased total distance traveled in the center (Fig. 3B) and fewer entries (Fig. 3C) into this area compared to the other groups. The worsening effects of BPA were not attributed to impaired locomotor activity, as the total distance traveled remained consistent across groups (Fig. 3D). Additionally, the average speed did not significantly differ among groups (Fig. 3E).

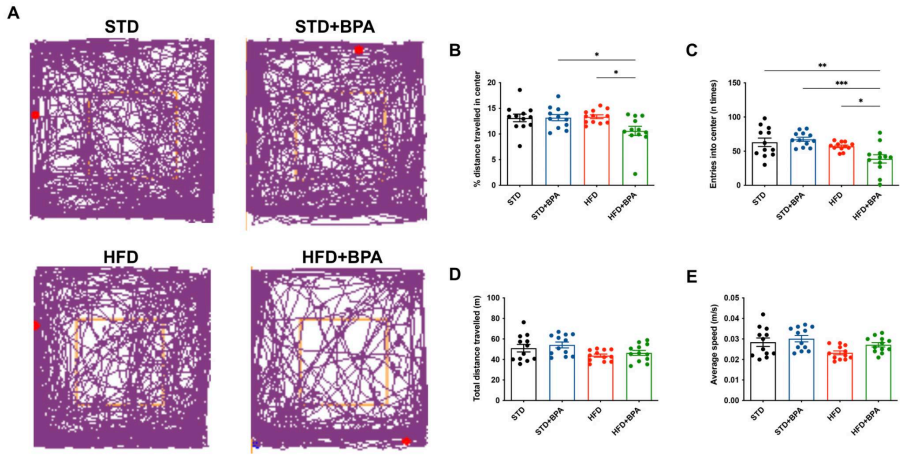

Fig. 3. BPA increases the anxiety-like behavior of obese mice. (A) Representative track records of all experimental groups during OFT, (B) Total distance in the center, (C) the number of entries into the center, (D) the total distance travelled, (E) average speed of mice. Data are presented as means ± SEM of all animals (n = 12 each group). Differences among groups were considered significant at least at values of *P < 0.05.

To further confirm the impact of BPA on anxiety-like behavior in obese mice, we conducted the EPMT (Fig. 4A), which evaluates anxiety-related aversion to open, elevated spaces. After 15 weeks of HFD feeding, obese mice spent less time (Fig. 4B) and had fewer entries (Fig. 4C) into the open arms compared to the STD mice. The anxiety-like behavior was exacerbated by BPA exposure, leading to an even greater reduction in time spent in the open arms. Like the OFT results, the anxiety-like behavior observed in BPA-exposed HFD mice was not associated with reduced locomotor activity, as indicated by total distance traveled and average speed (Fig. 4D and E). No significant differences in anxiety-like behavior were noted between the STD and STD + BPA groups, nor in the expression of mRNAs related to certain inflammatory and immune markers.

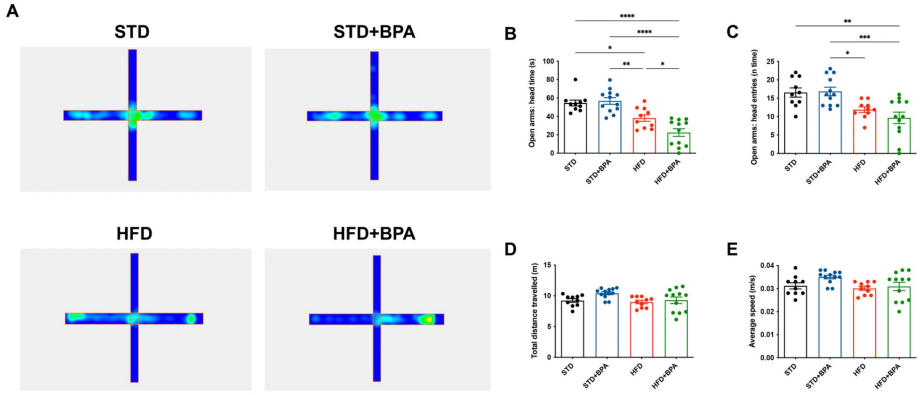

Fig. 4. BPA effects on the EPMT of STD and HFD mice. (A) Representative track records of all experimental groups during EPMT, (B) time and (C) number of entries open arms, (D) total distance travelled and (E) average speed. Data are presented as means ± SEM of all animals (n = 10–12 each group). Differences among groups were considered significant at least at values of *$P < 0.05$.

5.1.2 BPA exacerbates HFD-induced inflammation and immune activation in PFC of obese mice

Neuroinflammation is a key pathogenic mechanism underlying mood disorders associated with obesity. In our study, we analyzed the transcription of pro-inflammatory mediators, including TNFα and IL-1β, as well as the inflammasome NLRP3. BPA exposure significantly elevated the mRNA levels of TNFα and IL-1β, which were already heightened by the HFD (Fig. 5A and B). Additionally, BPA further promoted NLRP3 transcription in HFD mice (Fig. 5C). The neuroinflammatory response triggered by HFD was also linked to the activation of immune cells in the brain, prompting us to assess the mRNA levels of TLR4 and monocyte chemoattractant protein-1 (MCP-1). BPA notably increased the expression of these harmful factors compared to other groups (Fig. 5D and E).

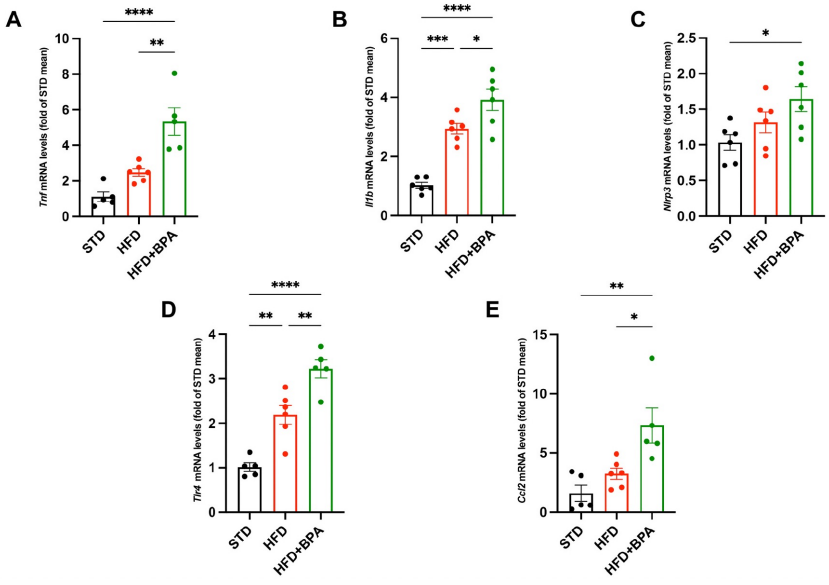

Fig. 5. BPA exacerbates HFD-induced inflammation in PFC of obese mice. The mRNA transcription of (A) *Tnf*, (B) *Il1b*, (C) *Nlrp3*, (D) *Tlr4*, and (E) *Ccl2* in the PFC of STD and HFD mice. Data are presented as means ± SEM of all groups of

mice (n = at least 5 each group). Differences among groups were considered significant at least at values of *$P < 0.05$.

5.1.3 BPA affects the HPA axis increasing mRNA expression of Crh and Crh receptor 1 in PFC

The involvement of the PFC in controlling stress-induced hyperactivation of the HPA axis has been highlighted by the elevated activation of corticotropin-releasing hormone receptor 1, which is expressed in this brain region. In this study, we showed that BPA elevated the mRNA levels of Crh and Crhr1, which were already increased by the HFD (Fig. 6A and B).

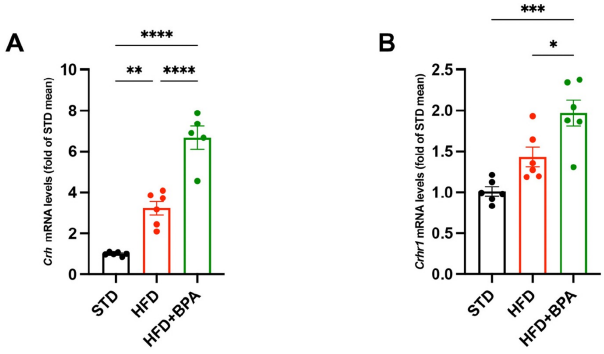

Fig. 6. BPA stimulates the HPA axis in obese mice. mRNA expression of (A) *Crh*, and (B) *Crhr1* in the PFC of all groups of animals. Data are presented as means ± SEM of all groups of mice (n = at least 5 each group). Differences among groups were considered significant at least at values of *P < 0.05.

5.1.4 BPA induces inflammation and immune response in hypothalamus and amygdala of obese mice

We further investigated the effects of BPA on the hypothalamus and amygdala in HFD-fed mice. After 15 weeks of HFD, there were no significant changes in the inflammatory mediators examined (TNFα and CCL2). However, in the HFD+BPA group, both mediators were significantly elevated in these brain areas (Fig. 7A, B, E, and F).

Notably, BPA also increased the expression of two specific murine must cell proteases, Tpsb2 and Cma1 (Fig. 7C and D).

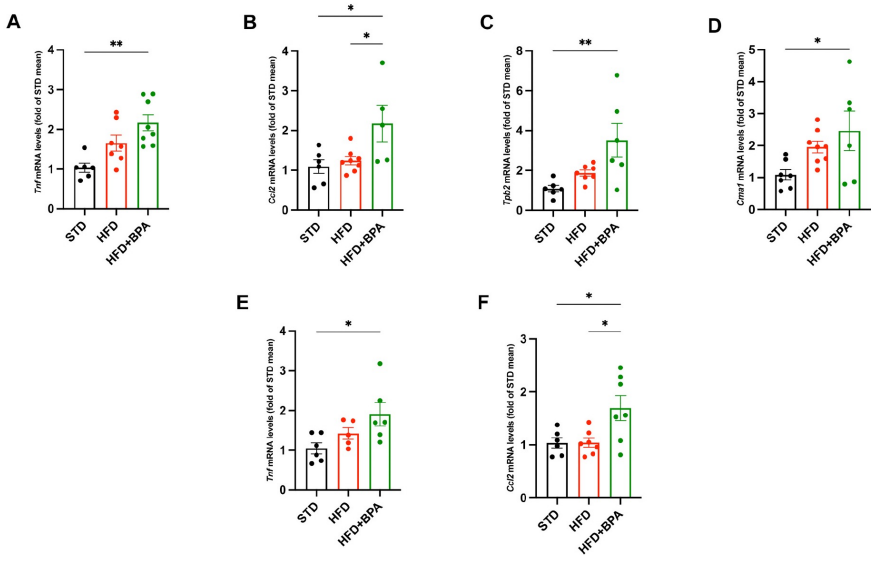

Fig. 7. BPA induces inflammation in hypothalamus and amygdala of HFD-fed mice. mRNA transcription levels of (A) *Tnf*, (B) *Ccl2*, (C) *Tpb2* and (D) *Cma1* in hypothalamus, mRNA expression of (E) *Tnf* and (F) *Ccl2*. in amygdala. Data are presented as means ± SEM of all groups of mice (n = at least 5 each group). Differences among groups were considered significant at least at values of *P < 0.05.

5.2 Effect of PEA-OXA on obese mice

5.2.1 PEA-OXA counteracts the anxiety-like behavior in obese mice

The effects of PEA-OXA on the anxiety-like behavior of obese mice were assessed by OFT and EPMT. The OFT showed that HFD group mostly travelled to the periphery of the arena and the treatment with PEA-OXA significantly enhanced the time (expressed in s) spent in the center (Fig. 8A). Conversely, a not significant increasing trend in the number of entries was observed (Fig. 8B). There were no differences among groups in the average speed and total distance travelled, excluding the impact of a locomotory impairment (Fig. 8C and D).

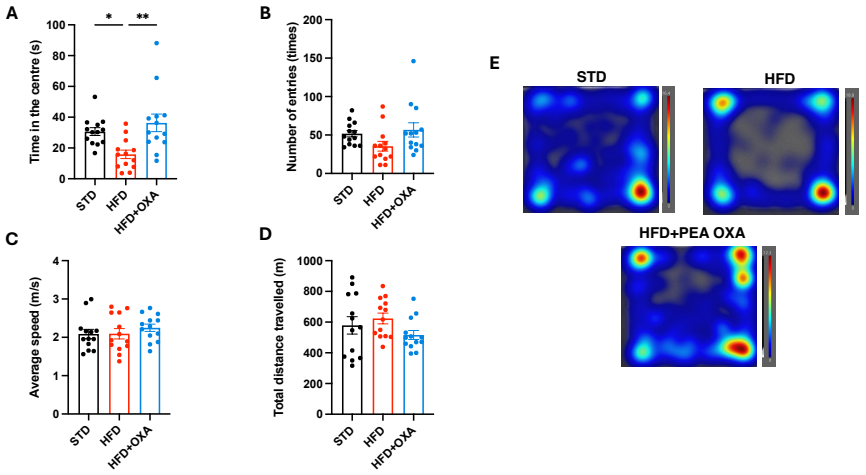

Figure 8. The time spent in the center of the arena (A), the number of entries in the same area (B), the average speed (C) and the total distance travelled (D) were analyzed in open field test to assess the anxiolytic activity of PEA-OXA. (E) A representative heatmap made during the test and showing the anxiolytic effects of PEA-OXA compared to the thigmotaxis expressed by obese mice. Data were showed as mean ± SEM (n= 13 each group) reaching the significance at $P < 0.05$

The EPMT confirmed the effects of PEA-OXA in counteracting anxiety-like behavior. Obese mice treated with PEA-OXA spent more

time and made more entries in open arms compared to HFD group (Fig. 9A and B).

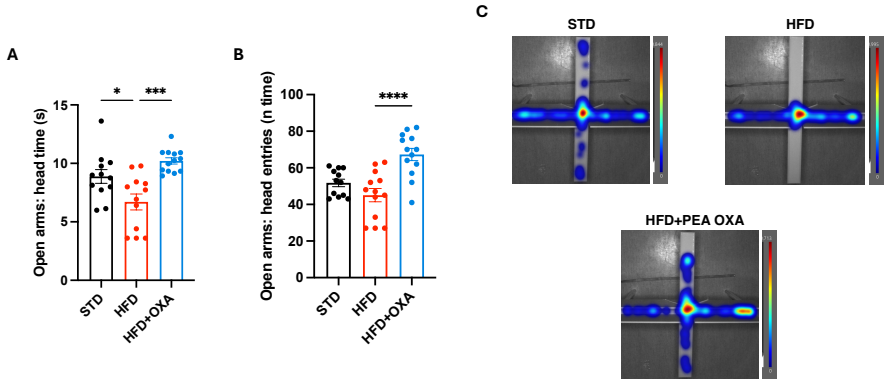

Figure 9. The anxyolitic properties of PEA-OXA were confirmed by Elevated Plus Maze test. Here we evaluated the time spent (A) and the number of entries in open arms (B). (C) Representative heatmap made during the test and showing the preference of untreated obese mice and HFD+OXA group for closed or open arms respectively. Data are shown as mean ± SEM (at least 12 mice per group) reaching the significance at $P < 0.05$.

5.2.2 PEA-OXA lessens neuroinflammation in hippocampus of obese mice

The immunomodulatory and inflammatory patterns were evaluated in the hippocampus of obese mice from all experimental groups. The HFD feeding enhanced the protein levels of TLR4 and COX-2 that were normalized by the treatment with PEA-OXA (Fig. 10A and B). Moreover, PEA-OXA countered the increase of NLRP3 and IL-1β, normalizing the transcriptional levels of these two inflammatory markers (Fig. 10C and D).

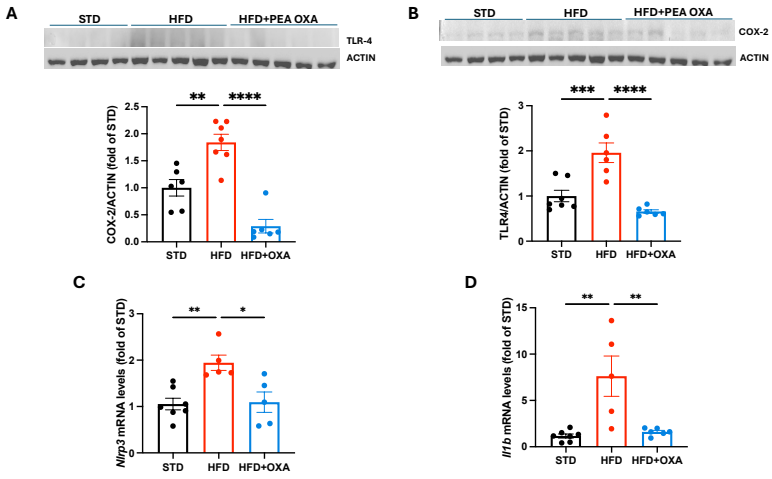

Figure 10. PEA-OXA counteracts inflammation in hippocampi of obese mice. Here we evaluated the protein expression of TLR4 (A) and COX-2 (B), two well-known inflammatory markers, by Western-Blot analyses. Moreover, the gene expressions of the inflammasome *Nlrp3* (C) and those of *Il-1β* (D) were assessed by Real-Time PCR. Data were showed as mean ± SEM (at least 5 animals each group) reaching the significance at $P < 0.05$.

5.2.3 PEA-OXA restores blood-brain barrier integrity in hippocampi of obese mice

It is well known that the HFD feeding can potentially compromise the integrity of the BBB. The transcriptional level of the tight junctions Cldn5 and Ocln, significantly decreased in untreated obese mice, was restored by PEA-OXA treatment, which exerted its ameliorating effect on BBB integrity (Fig. 11A and B).

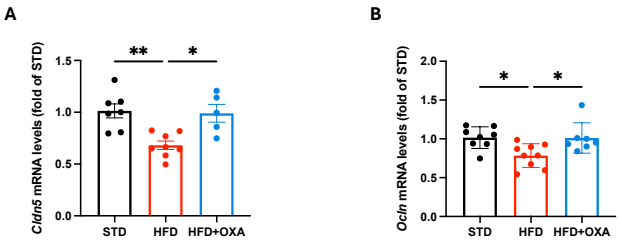

Figure 11. PEA-OXA improved BBB integrity compromised by HFD. The transcriptional levels of *Cldn5* (A) and *Ocln* (B), two of the main tight junctions in brain were evaluated in hippocampus from all experimental groups by Real-Time PCR. Data were showed as mean ± SEM (at least 5 mice per group) reaching the significance at $P < 0.05$.

5.2.4 Effects of PEA-OXA on hippocampal neurogenesis and synaptic plasticity

The gene expression of Dcx1, EGR1 (Figure 12A and B), markers related to the neurogenesis and synaptic plasticity, was evaluated in the hippocampus of the mice from all experimental groups. PEA-OXA-treated mice showed a normalized expression of these genes, that was compromised by the HFD. Moreover, besides there was no difference between STD and HFD groups, PEA-OXA promoted a significant increase in transcriptional level of mKi67 (Figure 12C).

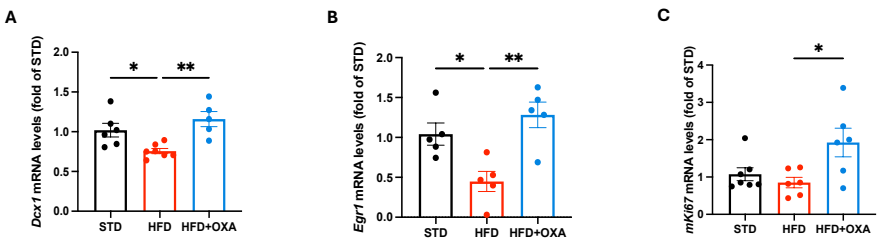

Figure 12. PEA-OXA restored neurogenesis in hippocampi of obese mice. Here we evaluated by Real-Time PCR the gene expression of *Dcx1* (A), *Egr1* (B) and *mKi67* (C), three important markers of neurogenesis in adult hippocampus. Data were showed as mean ± SEM (n=5-7 each group) reaching the significance at $P < 0.05$.

5.2.5 PEA-OXA counteracts unfold protein response (UPR) and insulin resistance in hippocampus of obese mice.

Following a chronic exposure to an HFD, the UPR can occur, activating endoplasmic reticulum (ER) stress. Here, we evaluated the protein expression of binding immunoglobulin protein BiP (Figure

13A) and the phosphorylation of eukaryotic translation initiation factor 2 subunit (eIF2)α (Figure 13B). PEA-OXA restored the protein levels of BiP, consequently limiting the phosphorylation of eIF2a.

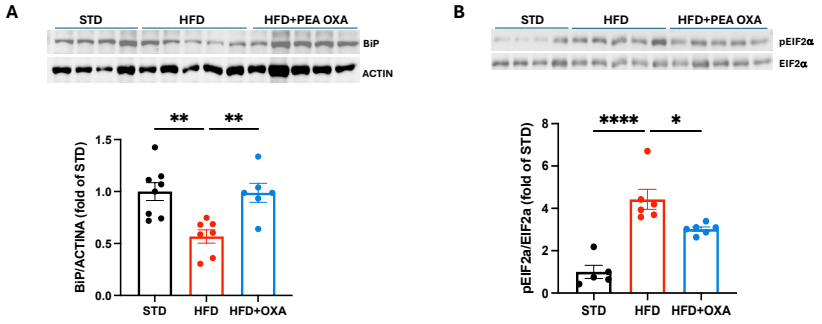

Figure 13. PEA-OXA prevents the endoplasmic reticulum stress activation in hippocampus. The protein expression of BiP (A) and the phosphorylation of EIF2α (B) were analyzed by Western-Blot. Data are shown as mean ± SEM (at elast 5 animals each group) reaching the significance at $P < 0.05$.

Moreover, the persistence of ER stress caused by the HFD can lead to an increase in the protein expression of PTP1B, related to the insulin resistance. PEA-OXA led to a decrease in the protein expression of PTP1B (Figure 14A), and an increase in the phosphorylation of protein kinase B (Akt) and Glycogen synthase kinase 3 (GSK3)β (Figure 14B and C).

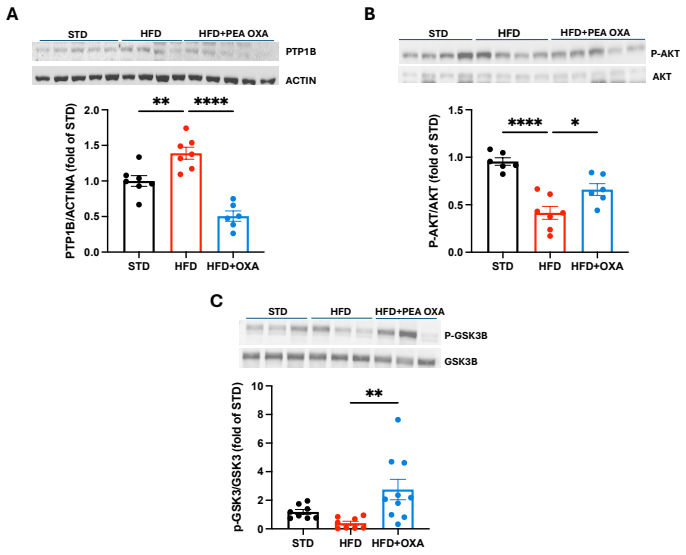

Figure 14. PEA-OXA restore the insulin signaling in HFD mice. The protein expression of PTP1B (A) and the phosphorylation of AKT (B) and GSK3β were analyzed by Western-Blot in hippocampus from all experimental groups. Data are shown as mean ± SEM (at least 4 animals per group) reaching the significance at $P < 0.05$.

5.3 Neuropsychiatric effects of neonatal LPS in early adult life

5.3.1 The impact of LPS injection on OCD-like behavior in both gender rats

Vehicle- and LPS-injected rats performed marble burying and self-grooming tests to evaluate stereotyped movements between PND 42 and 44 (Figure 15A). Female LPS rats significantly buried a higher number of marbles than female controls (Figure 15B), while no differences were highlighted in both vehicle and LPS-treated male rats. This stereotypic-like attitude of the female LPS group was confirmed by the self-grooming test, in which LPS-challenged female rats spent more time in grooming and licking than female vehicle-injected rats (Figure 15C).

We also monitored the total distance traveled, the freezing behavior (time and frequency), and the time of center crossings in the open-field arena. Both male and female LPS-injected rats were hyperactive and showed an increased anxiety-like behavior. Indeed, all LPS-injected rats traveled more distance than control groups without any freezing events. This restless behavior was accompanied by an increased thigmotaxis, determined by a reduced time spent in the center zone of the arena compared to Saline groups (Figure 15D).

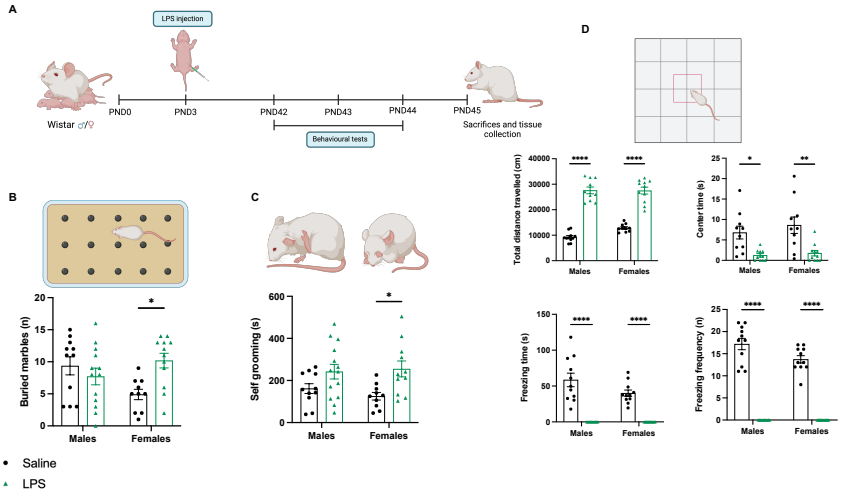

Figure 15. LPS induced stereotyped and repetitive behavior only in female rats and a strong hyperactivity in both genders. Male and female Saline rats were intraperitoneally injected with LPS (1 mg/kg) at PND3. Behavioral tests were carried out to assess alterations caused by the bacterial challenge between PND42 and PND44 (A) (at least 10 animals each group). The number of buried marbles was higher in the female LPS group compared to the control animals (B) while no changes were noticed in male rats. The effects and gender-related differences of the LPS in the stereotyped movement were then confirmed by self-grooming test (C). The total traveled distance, freezing time, freezing frequency and time spent in the center were the parameters analyzed during the open-field test to evaluate the effects of the LPS on hyperactivity and anxiety-like behavior (D). Data were showed as mean ± SEM reaching the significance at $P < 0.05$.

5.3.2 Post-natal LPS injection induces systemic pro-inflammatory and immune markers in rats

Inflammation and immune response induced by post-natal injection of LPS were evaluated in the serum of all experimental groups collected at PND45 by Bio-Plex Assay. The immune challenge augmented GM-CSF (Figure 16A), MIP-1α (Figure 16C), MCP-1 (Figure 16D), IL-1β (Figure 16F), and IL-7 (Figure 16G) in the serum of female LPS rats. Conversely, LPS caused an increase in M-CSF (Figure 16B) levels in only male rats. Moreover, Gro-KC (Figure 16E) levels were increased in both male and female LPS groups.

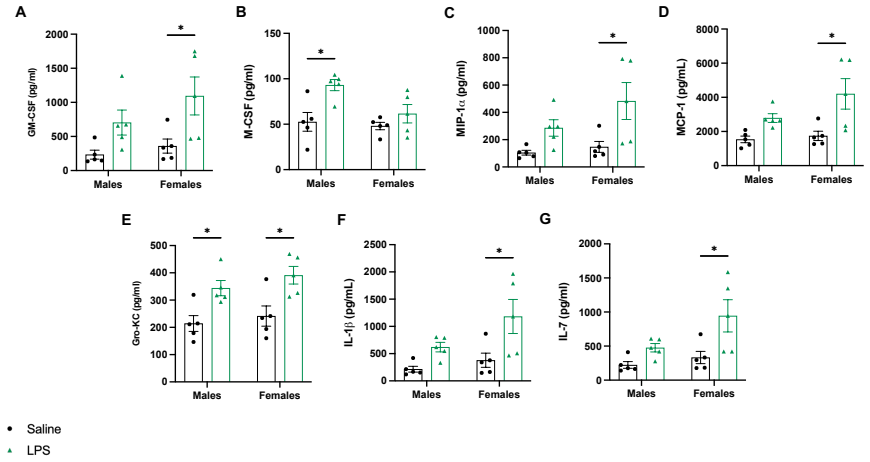

Figure 16. LPS triggered a systemic inflammation. The immunomodulatory markers GM-CSF (A), M-CSF (B), MIP-1α (C), MCP-1 (D), Gro-KC (E), and cytokines IL-1β (F), and IL-7 (G) were evaluated in serum from all groups by a 23-rat cytokines Bio-Plex assay (n= 5 animals each group). Data were expressed as mean ± SEM reaching the significance at $P < 0.05$.

5.3.3 The effects of LPS challenge on GABA and glutamate levels in different brain areas of rats

We evaluated the levels of GABA, DA, and 5-HT in PFC and striatum (Figure 17). Post-natal injection of LPS caused a significant reduction of GABA levels in PFC of female rats (Figure 17A). Otherwise, the immune challenge did not change the GABA levels in striatum (Figure 17D). Interestingly, besides the LPS challenge did not determine intragender differences regarding the DA levels in both PFC and striatum (Figure 17B and E), the female LPS group showed a significantly higher level of this neurotransmitter in striatum compared to the male challenged rats (Figure 17E). Moreover, the endotoxin promoted a significant reduction of 5-HT (Figure 17C) in PFC of female rats.

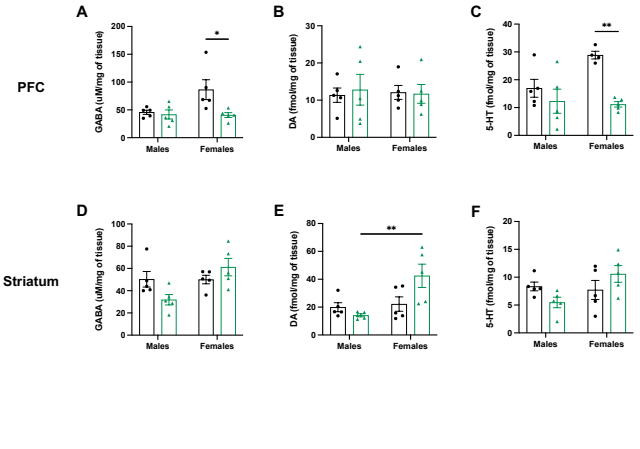

Figure 17. The infection with LPS unbalanced neurotransmitter levels in the prefrontal cortex and striatum of female rats. The levels of neurotransmitters γ-aminobutyric acid (GABA; A, D), dopamine (DA; B, E), and serotonin (5-HT; C, F) were measured in the prefrontal cortex (PFC), and striatum and of all rats by HPLC (n= 4-5 animals each group). Data were expressed as mean ± SEM reaching the significance at $P < 0.05$.

5.3.4 Neonatal infection triggers inflammation in cortico-striatal-thalamic circuit

The post-natal injection with LPS caused several changes in the mRNA expression of different markers related to the inflammation in the PFC and striatum. In PFC, although there were no statistical differences in the transcriptional levels of NLRP3 and NF-κB (Figure 18A and B) among all groups, male LPS-treated rats showed a significant increase in mRNA levels of TNF-α compared to male control group and LPS Female rats (Figure 18C). Meanwhile, the transcriptional levels of IL-1β were significantly enhanced only in the female LPS group compared to the same-gender vehicle animals (Figure 18D). In striatum, a similar gender profile for TNF-α and IL-1β was shown

(Figure 18G and H). No significant differences among groups were found in this brain area for NLRP3 and NF-κB (Figure 18E and F).

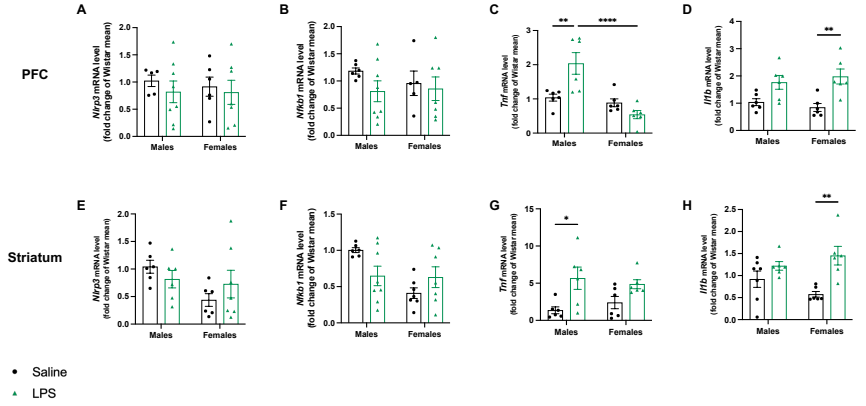

Figure 18. Neuroinflammation provoked neuroinflammation in cortico–striatal–thalamic circuitry. The gene expression of the inflammatory markers *Nlrp3* (A, E), *Nfkb1* (B, F), *Tnf* (C, G) and *Il1b* (D, H) were assessed in prefrontal cortex (PFC) and striatum of all experimental groups by Real-Time PCR (at least 5 animals each group). Data are expressed by mean ± SEM reaching the significance at $P < 0.05$.

5.3.5 LPS induces oxidative stress and lipid peroxidation in a gender-dependent manner

The possible damage of the cortico–striatal–thalamic circuitry induced by LPS has been triggered by oxidative stress. Indeed, we evaluated the levels of reactive species of oxygen (ROS) and of 4-hydroxynonenal (4-HNE), a critical marker of lipid peroxidation in specific brain areas previously analyzed (Figure 19). Interestingly, we demonstrated a gender difference, where ROS levels were higher in the striatum of only female LPS-injected than Saline group (Figure 19B). Otherwise, male LPS-injected rats showed a higher lipid peroxidation in the striatum and thalamus than control rats (Figure 19E and F), an event not evidenced in the female LPS group.

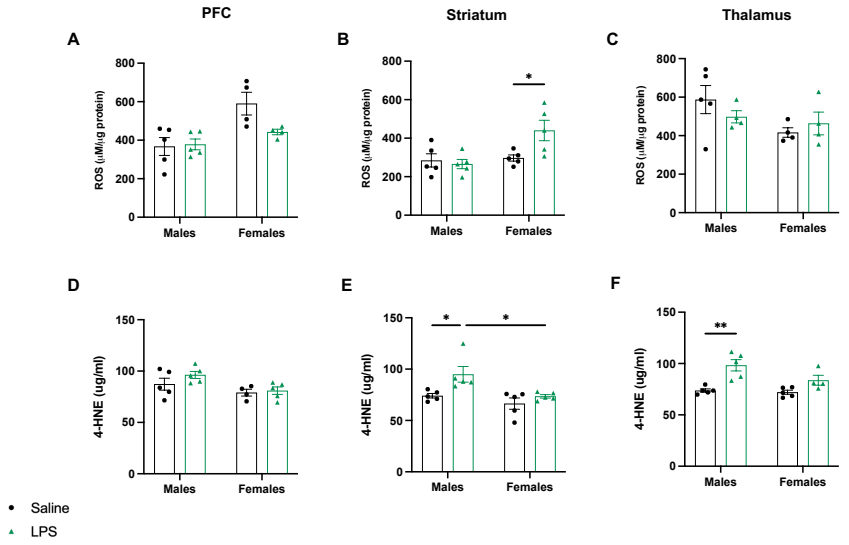

Figure 19. LPS injection caused an impairment in reactive oxygen species and lipid peroxidation in the cortico–striatal–thalamic circuit. The levels of the reactive oxygen species (ROS; A, B, C) and those of 4-hydroxynonenal (4-HNE; D, E, F), a critical marker of lipid peroxidation, were analyzed in the PFC, striatum, and thalamus of rats from all groups by HPLC (at least 4 animals each group). Data were expressed as mean ± SEM reaching the significance at $P < 0.05$.

6. Discussions

6.1. Worsening effect of Bisphenol A (BPA) on anxiety-like behavior of obese mice

The interplay among metabolic dysfunction, mood disorders, and exposure to endocrine-disrupting chemicals can perpetuate a detrimental cycle that exacerbates obesity and its associated health risks. Obesity is well-documented as a significant risk factor for various metabolic disturbances, chronic inflammatory conditions, non-communicable diseases, and mood disorders [151]. This study represents the first comprehensive investigation into how sub-chronic BPA exposure induces or exacerbates anxiety-like behavior in obese mice, as well as its impact on crh/crh1r system, neuroinflammation, and immune activation in the PFC, hypothalamus, and amygdala of obese mice. Based on current literature, we focused on analyzing BPA's effects on brain mechanisms linked to anxiety-like behaviors, particularly in male animals which are more susceptible [152,153].

Individuals with obesity and diet-induced obese rodents exhibit anxiety-like behaviors [73,154], largely attributed to dysregulation of the HPA axis and neuroinflammation. Previous studies have shown that perinatal or adolescent BPA exposure significantly affects anxiety- and depressive-like behaviors [155,156]. In our study, the open field test (OFT) was performed to assess exploratory behavior, general activity, and fear responses, while the elevated plus maze test (EPMT) was employed as a standard measure for anxiety [157,158]. Interestingly, under our experimental conditions, the total distance traveled in the central zone and the number of entries into this area did not differ between obese and control lean mice. However, BPA-exposed obese mice predominantly traveled along the arena's periphery, indicating

reduced exploration behavior. BPA-treated obese mice showed a diminished inclination to explore central areas, perceived as less secure, compared to untreated obese mice. Likewise, BPA exposure decreased the time spent in the open arms during the EPMT, a reduction already observed in untreated obese animals. These findings highlight BPA's exacerbating effect on anxiety-like traits induced by HFD and its impact on exploratory behavior.

Previous research by Wise and Hernandez-Saavedra [159] found that concurrent perinatal BPA and HFD exposure in rats did not significantly affect anxiety-like behaviors, though maternal care and offspring behaviors were influenced. The authors suggested that BPA might alter the long-term structure of neural regions, such as the medial PFC, affecting behavior only weeks later. Additionally, prenatal or perinatal BPA exposure in C57Bl/6J male mice resulted in increased anxiety-like behavior in the OFT compared to controls, with a pronounced effect in males versus females [156,160,161]. Recent studies have shown that oral BPA (50 μg/kg) exacerbates immune, metabolic, and mitochondrial dysfunction in the liver of male obese mice, impacting glucose and lipid metabolism and activating oxidative stress and inflammasome pathways [162]. BPA's effect in enhancing systemic MCP-1 and portal LPS levels, alongside decreased IL-10, underscores its detrimental immunological effects in HFD-fed mice.

Ma and Deng [163] recently demonstrated that long-term (12 weeks) low-dose BPA exposure worsens diet-induced prediabetes in both male and female mice. Their study established that TLR4 is essential for BPA-induced hypothalamic inflammation and astrocyte activation, as TLR4 deficiency mitigates BPA's adverse effects on prediabetes. Despite widespread concerns about BPA's impact on obesity and

related comorbidities, the precise molecular mechanisms remain unclear. Prolonged HFD feeding leads to metainflammation and subsequent neuroinflammation, with the hypothalamus being a primary focus [164]. Excessive fatty acid flux induces hypothalamic inflammation and dysfunction, which is characterized by microglia activation, increased TLR4 expression [165], and elevated cytokine levels [166,167]. The neuroinflammatory hypothesis suggests that inflammation contributes to depression and anxiety, with the hypothalamus influencing other mood-regulating brain areas such as the hippocampus, cortex, and amygdala [168].

In our study, BPA exposure led to significant increase in TNFα and IL-1β transcription in the PFC of obese mice, as well as NLRP3 inflammasome activation, which was not modified by 15 weeks of HFD feeding. Additionally, BPA significantly raised TLR4 and MCP-1 levels in the PFC compared to untreated obese mice. Previous studies have shown that HFD feeding activates cortical TLR4 and NF-kB, leading to overexpression of proinflammatory cytokines and chemokines [169,170], along with cortical astrocyte and microglia activation [168]. Pistell et al. [171] found that HFD, but not a western diet, triggers cortical neuroinflammation, highlighting HFD's detrimental effects.

In our study, 3 weeks of BPA exposure exacerbated inflammatory parameters and associated health risks, akin to effects observed in metabolic organs like the liver [162]. Obesity-related HPA axis dysregulation [151,168] contributes to anxiety pathogenesis [171], with neuroinflammation affecting hypothalamic communication with other mood-regulating brain regions and increasing Crh release [172]. Our data reveal elevated transcription of Crh and its receptor crhr1 in the

PFC of obese mice, further increased by BPA exposure. Notably, Crh-R1 null mutants and Crh-R1 mRNA antisense probe-treated rats exhibited reduced anxiety-like behavior [173,174], while Crh-R1 is upregulated in the amygdala following chronic stress [175]. Although Crh-R1 receptors in the PFC are involved in stress responses, specific evidence regarding BPA's impact on obesity and HPA regulation is lacking.

Finally, we assessed inflammation in the hypothalamus and amygdala of obese mice following BPA exposure. While 15 weeks of HFD alone did not significantly alter inflammatory mediators, BPA treatment increased TNFα and MCP-1 in both regions. Notably, BPA also elevated the transcription of two specific mast cell proteases, Cma1 and Tpsb2, in the hypothalamus. Mast cells, which can cross the BBB when disrupted [60], play a role in the initial stages of neuroinflammation and precede glial activation [176]. In summary, our study demonstrates that BPA exacerbates obesity-induced anxiety-like behavior in male mice through alterations in the HPA axis, neuroinflammation, and immune activation in the PFC, with early involvement of immune cells in the hypothalamus and amygdala.

6.2 Effect of PEA-OXA in limiting neuroinflammation and anxiety-like behavior of obese mice

Besides being a risk factor for other comorbidities, such as cardiovascular diseases, type 2 diabetes and cancer, obesity is often associated with a high prevalence of mood disorders [10]. Individuals with obesity exhibiting elevated inflammation are more likely to meet criteria for metabolic syndrome and to develop mood disorders (i.e. Major Depressive Disorder and anxiety) [177]. Under metabolic stress, adipocytes produce chemoattractant molecules and inflammatory mediators, first leading to metainflammation and then to the onset of neuroinflammation. This inflammatory process is characterized by the activation of microglia cells and astrocyte and can potentially affect brain areas involved in mood regulation [178]. The contribution of neuroinflammation to the establishment of neuropsychiatric disorder has been widely demonstrated and animal models of HFD feeding have already pinpointed how the inflammatory process associated with obesity may be involved in peripheral and central disorders. The present study highlights the effect of PEA-OXA, an oxazoline of Palmitoylethanolamide (PEA), in counteracting the anxiety-like behavior caused by high-fat diet feeding. Likewise, individuals suffering from anxiety disorders can exhibit elevated circulating levels of inflammatory markers, including CRP, IL-1β, IL-6, and TNFα [168]. Additional evidence contributing to neuroimmune theories of depression and anxiety arises from observations that inflammatory interventions, e.g. interferon IFNα treatment, can lead to symptoms of these disorders [179]. Obese rodents exhibit anxiety-like behavior, largely attributed to dysregulation of the HPA axis and neuroinflammation [6]. Here, we performed the OFT to assess the

effects of PEA-OXA on thigmotaxis of obese mice and EPMT, considered as one of most specific tests for investigating anxiety-like behavior in rodents. Interestingly, PEA-OXA treatment promoted an increase in time spent in the center of the arena in obese mice while only a not significant increasing trend was observed in the number of entries. To exclude an impairment in locomotor activity on the effect of PEA-OXA, the total distance traveled, and the average speed were measured evidencing the absence of change in these parameters. The EPMT confirmed the anxiolytic effects of PEA-OXA. Indeed, the oxazoline prompted the obese mice to enhance the number of entries and the time spent in the open arms.

The effects of diet-induced obesity on the hippocampus have received considerable attention because of the well-reported effects of HFD to elicit cognitive and emotional impairments [180]. Among them, neurogenesis is disrupted in the hippocampus, a key structural alteration associated with depression and antidepressant responses. High-fat feeding triggers hippocampal proinflammatory marker upregulation that can elicit anxiety-like behavior, reduce neurogenesis, and impair hippocampal function. Moreover, The intake of saturated fatty acid based diet and the increased erythrocyte content of saturated fatty acids (indicator of long-term consumption) contribute to inflammation by favoring TLR4 signaling in macrophages [181]. In this context, PEA-OXA not only reduced the expression of TLR4, but also the levels of the COX-2, IL-1β, and NLRP3 in hippocampus of obese mice, indicating its marked anti-inflammatory properties. Moreover, obesity-associated low-grade inflammation induced a significant BBB disruption in the hippocampus of mice [14]. The underlying mechanisms are likely multifaceted and may involve increased

endothelial oxidative stress [182], pericyte dysfunction, and changes in tight junctions, which form an essential structural component of the BBB [14]. The integrity of the BBB is central to neural health, and many neuropsychiatric disorders are associated with its breakdown [183]. In our experimental condition, the lack of BBB integrity caused by HFD feeding was prevented by PEA-OXA, which normalized in the hippocampus of obese mice the expression of Cldn5 and Ocln, two of the main tight-junctions in CNS.

Adult hippocampal neurogenesis is involved in memory and learning, indeed disrupted neurogenesis is implicated in cognitive impairment and mood disorders, including anxiety and depression [184]. Pro-inflammatory cytokines released in the periphery are involved in peripheral immune system-to-brain communication by activating resident microglia in CNS [138]. Activated microglia reduce neurogenesis by suppressing neuronal stem cell proliferation, increasing apoptosis of neuronal progenitor cells, and decreasing survival of newly developing neurons and their integration into existing neuronal circuits [142]. The obese phenotype and neuroinflammation are conditions associated with behavioral disturbances linked to disrupted adult hippocampal neurogenesis. Several studies have pinpointed the role of Egr1 in hippocampal neurogenesis [185]. This gene is rapidly induced in association with LTP and in behaviorally relevant brain structures and circuits after specific learning experience [185]. Our data evidenced a reduction of the levels of Egr1 by HFD feeding, that was significantly reverted by PEA-OXA. Moreover, we analyzed the levels of Dcx1 and mKi67, two important endogenous markers related to adult neurogenesis and synaptic plasticity. PEA-OXA counteracted the reduction driven by the

HFD-feeding on the Dcx1 and mKi67 levels in the hippocampus, indicating the neuroprotective effects of the PEA-OXA in obese mice. Besides the lack of integrity of BBB and altered neurogenesis and synaptic plasticity, along with neuroinflammation, the Unfold Protein Response (UPR) can occur [186]. UPR globally results in the ER stress and some studies has evidenced the role of ER stress in worsening the anxiety-like behavior in mice, focusing on the causative role of the UPR on abnormal apoptosis of brain cells [181].

In the early stage of ER stress, the Glucose-regulated protein 78, GRP78 is bound to the unfolded or misfolded protein, thereby reducing protein synthesis, increasing protein degradation and promoting physiological protein folding to relieve ER stress [187]. One of the three ER stress pathway starts with BiP removal and the phosphorylation of eIF2α, leading to neuronal apoptosis [187]. Our data evidenced that untreated obese mice exhibit at hippocampal level a lack in BiP protein expression and an enhanced phosphorylation of eIF2α. PEA-OXA normalized the expression of these two proteins blocking in this way the signaling pathway. Moreover, a recent study showed that protein tyrosine phosphatase 1B (PTP1B) is strictly correlated to ER stress [188,189]. This protein is broadly expressed in various cells and tissues and has a pivotal role in decreasing the phosphorylation of the insulin receptor (IR) resulting in insulin resistance [190]. In individuals with obesity, decreased insulin sensitivity significantly correlates with greater depression and anxiety symptomology [191]. In support of this, reduced insulin signaling in brain regions controlling mood has been observed in animal models of obesity [192]. These findings suggest that impairments in insulin signaling modify the brain networks controlling mood and raise the question of whether insulin resistance contributes

to mood disorders. Consistently, inflammatory cytokines are a major contributor to insulin resistance: elevated circulating insulin activates macrophages and can promote insulin resistance [193]. In our experiment, PEA-OXA not only reduced the expression of PTP1B, but also improved the phosphorylation of p-Akt and p-GSK3β, demonstrating its potential in re-establishing the insulin signaling in the hippocampus of obese mice.

In conclusion, our study highlighted the beneficial effects of PEA-OXA on the anxiety-like behavior in a murine model of high-fat diet induced obesity. This compound demonstrated its capability in counteracting those molecular mechanisms that are involved in the link between obesity and anxiety, such as neuroinflammation, ER stress, insulin resistance and in restoring physiological neurogenesis and integrity of the BBB. However, further studies are required to completely understand all the mechanisms underlying the therapeutic potential of PEA-OXA.

6.3 Long-term neuropsychiatric effects of early exposure of LPS in rats

Transient perturbations, including infections, during the critical periods of neuronal maturation, trigger persistent changes in brain architecture possibly leading to the onset of neuropsychiatric alterations later in life [194].

Our study explored the direct relationship between early post-natal LPS challenge (at PND3) and long-term OCD- and schizophrenia-like behavior in rats (at PND45). We also investigated gender differences in developing specific behavioral abnormalities, neurotransmitter unbalance, inflammation and immune response activation, and oxidative stress in brain areas (PFC, striatum, and thalamus) associated to those neuropsychiatric disorders.

The obsessions are anxiety-provoking intrusive and persistent thoughts, uniquely associated with the human being as described in DSM-5 [16], and the range of compulsions in response to an obsession is wide, i.e., repetitive cleaning or washing, hoarding, checking, etc. Compulsive behavior in animals is characterized by excessive self-grooming and increased buried marbles during specific behavioral tests. The rodent stereotypy has been correlated with hyperactivity of DA signaling in the cortico-striatal pathway, particularly due to D1-expressing neuron [195]. In our experimental condition, we demonstrated, for the first time, a female-oriented susceptibility in stereotyped behavior, as well as the increase of striatal DA in LPS-injected rats. Indeed, D1 receptor knockout mice significantly reduce self-grooming occurences, indicating that D1R signaling in the striatum is involved in grooming behavior [196]. Interestingly, the rodent stereotypy is accompanied by hyperlocomotion and anxiety-like

behavior in schizophrenia-like animal models [197]. Consistently, we found that both LPS-injected male and female rats showed hyperactivity and anxiety-like alterations. In line with our findings, prenatal exposure to LPS increased locomotor activity at PND33 and PND60 without any sex-dependent differences [198]. Moreover, it was shown that the injection of poly I:C during early and late pregnancy in female mice caused strong hyperlocomotion and anxiety-like behaviors in male pups, while females were not evaluated [199]. Here, we confirmed not only schizophrenia-like behavior of both LPS-injected male and female rats, but also a female-specific reduction of GABA levels in PFC, already evidenced by Wischhof et al.[198]. Indeed, LPS-injected female rats showed reduced 5-HT as well as GABA levels in PFC, without any change in glutamate levels, thus indicating some possible sex-dependent alterations in both serotonergic pathways and excitatory-inhibitory balance. Supporting our observations, such neurochemical dysfunctions have been related to the onset of neurodevelopmental disorders, including ASD and schizophrenia [200]. Moreover, it has been reported that cortical glutamatergic and GABAergic transmissions are modulated by other neurochemical pathways, such as the dopaminergic and serotonergic ones, therefore contributing to the worsening of excitation/inhibition unbalance [201]. In addition, behavioral abnormalities induced by maternal immune activation have been associated with reduced number of parvalbumin-expressing thalamic and cortical GABAergic interneurons [199].

Given the nature of the challenge, the behavioral abnormalities induced by LPS are also strictly associated with a dysregulation of the immune system. Indeed, LPS triggers immune response through TLR4,

stimulating NF-kB-induced activation and releasing pro-inflammatory cytokines and chemokines [202]. Several preclinical studies have investigated the effects of single and repeated injections of LPS at different doses and at various time points (i.e., prenatal, postnatal, or adulthood) with contradictory or not fully clarified results. Furthermore, most of these studies evaluate the effects of pre- or post-natal challenges associated with other experimental conditions that sustain immune alteration, i.e. maternal separation or high-fat diet [203,204]. Data reported that triple injection of LPS (0,40 mg/kg, i.p.) at GD8, GD10, and GD12 caused no change in serum TNFα and IL-6 levels in 3-month-old pups (regardless of gender) [149]. Recently, Rojas et al. [127] measured serum levels of TNF-α at 6 h, 12 h, 24 h and 48 h after postnatal injection with LPS (PND7, 1 mg/kg, i.p.), evidencing a single peak at 6 h which decreased in a short-term time-dependent manner. In another preclinical study, male pups injected with LPS (100 μg/kg, i.p.) at PND14 caused a 3-hour plasmatic peak of inflammatory cytokines IL-1β, TNF-α, IL-6, and IL-10 as anti-inflammatory response [205]. However, these studies did not consider long-term immune modifications induced by LPS. Here, for the first time, we evaluated a wide range of serum cytokines and chemokines, evidencing that systemic inflammation and immune hyperactivation are still evident 42 days after a single LPS injection in both male and female insulted rats. Furthermore, these activations are clearer in females, resulting from higher serum levels of GM-CSF, some chemokines (GRO-KC, MIP1-α, MCP-1), and cytokines (IL-1β, and IL-7), while only M-CSF was higher in males than in females. During sepsis, high levels of GM-CSF, thought to be mainly due to macrophage activation, stimulate pro-inflammatory cytokines,

including IL-1β, IL-12, and IL-23, and induce tissue damage [206]. After crossing the BBB and blood-spinal cord barrier, GM-CSF priming activates LPS-induced NF-κB and the following production of pro-inflammatory cytokines via microglia [207]. The immune cell recruitment is also enhanced by chemokines, whose detrimental effects on the brain contribute to neurodevelopmental disorders, such as ASD and schizophrenia [208,209]. Schizophrenic patients show high basal levels of serum cytokines (i.e. IL-1, IL-6, IL-7), chemokines (MCP-1, MIP-1α, RANTES), and specifically GM-CSF [210]. Interestingly, multiple sex-based differences have been recently reported in schizophrenic patients: females show a tardive onset of the pathology with more severe positive symptoms [211], while the incidence is slightly higher in males with negative symptomatology [212].

The elevated levels of circulating inflammatory and immune mediators reverberate on the cortico-striatal-thalamic pathway. Interestingly, we found high levels of IL-1β in the PFC and striatum of females associated with serum cytokine increase and TNF-α in the same brain areas of males after 42 days of LPS injection without significant difference of serum TNF-α in both sexes.

The damage induced by inflammation and central immune cell activation leads to an excessive production of free radicals in the brain. In the present research, we showed that post-natal exposure to LPS increased ROS levels in the striatum of female rats and caused lipid peroxidation (4-HNE) in the striatum and thalamus of male rats after 42 days from immune activation. CNS structures are particularly vulnerable to oxidative stress, [71], because of their significant oxygen consumption [213], low functioning of antioxidant pathways, and an important amount of redox-sensitive cell types [214]. Interestingly,

GABAergic PV-positive interneurons are more vulnerable to oxidative stress during their maturation process in the postnatal period [215,216]. In addition, increased oxidative stress has been reported to crucially contribute to the onset and progression of several neuropsychiatric syndromes, including those related to infections occurring during pre- and post-natal life [217]. In fact, gestational exposure to poly:IC boosted oxidative stress in the brains of GD17-exposed female fetuses [218]. Consistently, Huang et al. [219] demonstrated increased levels of ROS and NO, as well as their producing enzymes, such as inducible nitric oxide synthases (iNOS) and NADPH oxidase (NOX)2, in the brains of pups born from LPS-injected dams.

This study confirmed the strict relationship between neonatal infection and neuropsychiatric disorders, including schizophrenia and OCD. Unlike most preclinical studies, we show the long-term impact of immune activation on the cortico-striatal-thalamic pathway considering sex differences. Furthermore, our data strictly demonstrates the importance of considering sex/gender in these types of evaluations. Indeed, we highlighted that female rats were more susceptible to post-natal exposure to LPS, evidencing more marked traits in the behavioral alterations and inflammatory response typical of PANS.

7. Conclusions

1. BPA exacerbates obesity-induced anxiety-like behavior and neuroinflammation via the HPA axis and immune activation

This study underscores the significant role of BPA in amplifying obesity-induced anxiety-like behaviors through its profound impact on the HPA axis, neuroinflammation, and immune activation, particularly within the prefrontal cortex (PFC), hypothalamus, and amygdala. Our findings reveal that short-term BPA exposure exacerbates both behavioral and molecular disruptions linked to anxiety, with heightened CRH/CRH-R1 transcription and involvement of the NLRP3 inflammasome in the PFC. Additionally, BPA-induced upregulation of pro-inflammatory cytokines and chemokines, alongside mast cell involvement in the hypothalamus, provides novel insights into the early immune responses contributing to neuroinflammation. These results highlight the complex interplay between metabolic dysfunction, environmental stressors like BPA, and mood regulation, further complicating the pathophysiology of obesity and its related comorbidities. The data call for further exploration of the precise mechanisms by which BPA impacts central nervous system structures and stress-regulating pathways.

2. Therapeutic potential of PEA-OXA in mitigating obesity-induced anxiety and neuroinflammation

This study highlights the promising therapeutic effects of PEA-OXA in alleviating anxiety-like behaviors associated with high-fat diet (HFD)-induced obesity. PEA-OXA demonstrated its efficacy in targeting key mechanisms underlying the interplay between obesity and anxiety, including neuroinflammation, BBB disruption, ER stress,

and insulin resistance. Our findings revealed that PEA-OXA reduced hippocampal inflammation by downregulating TLR4, COX-2, IL-1β, and NLRP3 expression, and preserving BBB integrity through the normalization of CLDN5 and OCLN. Furthermore, PEA-OXA reversed HFD-induced impairment in adult neurogenesis by restoring EGR1, DCX1, and mKi67 levels, suggesting a neuroprotective role by promoting synaptic plasticity. The compound also mitigated ER stress by normalizing BiP expression and reducing eIF2α phosphorylation, thereby preventing neuronal damage. Additionally, PEA-OXA enhanced insulin signaling by lowering PTP1B and improving p-Akt and p-GSK3β phosphorylation, underscoring its potential in counteracting insulin resistance—a known contributor to mood disorders in obesity. Since PEA-OXA showed robust effects in our model, further research is needed to fully elucidate its mechanisms and therapeutic potential in the treatment of obesity-linked anxiety and other neuropsychiatric disorders.

3. Long-term impact of neonatal infections on neurodevelopment and gender-specific vulnerability

This study provides critical insights into the lasting effects of neonatal infections on neurodevelopment, specifically focusing on postnatal LPS exposure and its contribution to neuropsychiatric disorders such as schizophrenia and OCD. Our findings highlight the detrimental role of immune activation, persistent inflammation, and oxidative stress in the CNS, with pronounced gender-specific vulnerabilities. Female rats exhibited greater susceptibility to stereotyped behaviors, GABAergic dysfunction, and sustained pro-inflammatory cytokine production, particularly in the prefrontal cortex and striatum. These results

underscore the relevance of excitatory/inhibitory imbalance and immune dysregulation in the pathophysiology of neurodevelopmental disorders. The persistent oxidative stress observed in key brain regions, coupled with gender differences in both behavioral and inflammatory responses, calls for further exploration into the mechanisms underlying these effects. Understanding the long-term consequences of early-life immune challenges is crucial for developing targeted therapeutic strategies to mitigate the risk of neuropsychiatric disorders associated with neonatal infections.

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