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REVIEW

Flavins and Flavoproteins in the Neuroimmune Landscape of Stress Sensitization and Major Depressive Disorder

Matt Scott Schrier[1], Maria Igorevna Smirnova[2–4], Daniel Paul Nemeth[1], Richard Carlton Deth[5], Ning Quan[1,3]

[1]Department of Biomedical Science, Charles E. Schmidt College of Medicine, Florida Atlantic University, Jupiter, FL, USA; [2]The International Max Planck Research School (IMPRS) for Synapses and Circuits, Jupiter, FL, USA; [3]Stiles-Nicholson Brain Institute, Florida Atlantic University, Jupiter, FL, USA; [4]Department of Biological Sciences, Charles E. Schmidt College of Science, Florida Atlantic University, Jupiter, FL, USA; [5]Department of Pharmaceutical Sciences, Barry and Judy Silverman College of Pharmacy, Nova Southeastern University, Ft. Lauderdale, FL, USA

Correspondence: Matt Scott Schrier, Florida Atlantic University, Building: MC-17, Room: 229E, 5353 Parkside Drive, Jupiter, FL, 33458, USA, Email mschrier@health.fau.edu

Abstract: Major Depressive Disorder (MDD) is a common and severe neuropsychiatric condition resulting in irregular alterations in affect, mood, and cognition. Besides the well-studied neurotransmission-related etiologies of MDD, several biological systems and phenomena, such as the hypothalamic-pituitary-adrenal (HPA) axis, reactive oxygen species (ROS) production, and cytokine signaling, have been implicated as being altered and contributing to depressive symptoms. However, the manner in which these factors interact with each other to induce their effects on MDD development has been less clear, but is beginning to be understood. Flavins are potent biomolecules that regulate many redox activities, including ROS generation and energy production. Studies have found that circulating flavin levels are modulated during stress and MDD. Flavins are also known for their importance in immune responses. This review offers a unique perspective that considers the redox-active cofactors, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), as vital substrates for linking MDD-related maladaptive processes together, by permitting stress-induced enhancement of microglial interleukin-1 beta (IL-1β) signaling.

Keywords: cofactor, cytokines, IL-1β, microglia, neuroinflammation, redox

Introduction

General introduction

Major depressive disorder (MDD) is linked with an increased risk of suicide, which claimed over 45,000 lives in the United States in 2020.[1] MDD is also reported to increase the risk of other neurological disorders, including Alzheimer's disease,[2,3] as well as increasing overall mortality risk.[4] For decades, MDD treatment has focused on antidepressant drugs that increase levels of extracellular monoamines, especially serotonin, in the brain. These drugs, such as selective serotonin reuptake inhibitors (SSRIs), are usually helpful for those with MDD[5] and may decrease suicide risk.[6] However, the effectiveness of current antidepressants is limited, and a substantial percentage of individuals with MDD do not obtain adequate relief from treatment.[7]

Involvement of the immune system in depression and in antidepressant resistance has gained increased attention in recent years. The immune system conveys messages to other cell types in the body through many mediators, such as cytokines. Some of the first evidence for a cytokine-mediated etiology of depressive symptoms came to light when treatment of healthy controls or cancer patients without a history of depression with Interleukin-2 or Interferon-alpha led to depressive symptoms.[8,9] These data suggested that immune mediators, specifically cytokines, are important for the generation of depressive symptoms. Interleukin-1 beta (IL-1β) is one of the primary cytokines produced and released in response to stressors.[10,11] Interestingly, elevated serum IL-1β levels have been found in some patients with MDD[12] and

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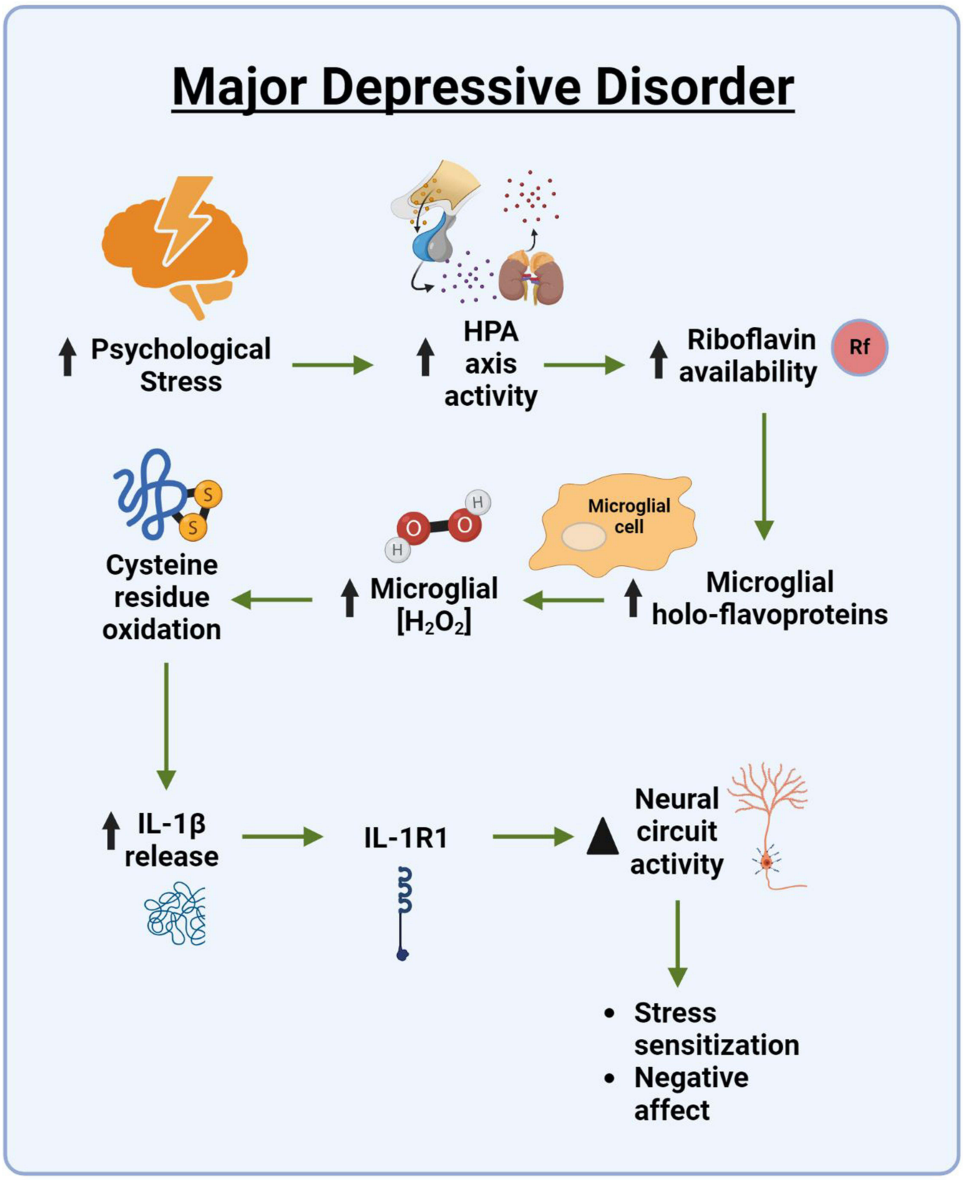
Graphical Abstract



pre-clinical studies support a causal role for IL-1β in the induction of depression-like phenotypes in rodents.[13–15] Basic knowledge of the physiological activities of IL-1β is key for understanding its implications for MDD development. IL-1β transmits pro-inflammatory messages via binding to and activating interleukin-1 receptor type I (IL-1R1),[16] expressed on the surface of immune, endothelial, and other cell types. In leukocytes, IL-1R1 activation increases transcription and subsequent translation of a variety of cytokines, chemokines, and enzymes. In this manner, IL-1β/IL-1R1 coordinates the immune response to infection. In addition, IL-1R1 is expressed by a variety of peripheral tissues[17] and cell types in the brain,[18,19] including neurons. Neuronal IL-1R1 has been thought to regulate affect, learning, memory, and certain behaviors.[20]

The presence of IL-1R1 throughout the body and brain enables IL-1β to influence neuronal activity by interacting with several systems that also have neuromodulatory effects. For example, IL-1β/IL-1R1 signaling in dorsal raphe nucleus neurons has long been associated with regulation of the brain serotonin system.[21–24] IL-1β/IL-1R1 signaling also

engages in important cross-talk with the hypothalamic-pituitary-adrenal (HPA) axis, stimulates formation of reactive oxygen and nitrogen species (ROS and RNS, respectively), and decreases mitochondrial function. Most of the systems that IL-1β modifies, can also feedback to regulate IL-1β. These relationships form a coordinated web of interacting systems, which endow organisms with the ability to respond to a multitude of stimuli while maintaining homeostasis under either normal or adverse conditions. However, deficits in metabolic and nutritional factors can lead to dysfunction in these sophisticated networks, contributing to MDD.

Vitamins and minerals are critical for the function of many proteins and enzymes required by animal species for survival. Riboflavin (Rf), more commonly known as Vitamin B2, is an inactive precursor to the enzymatic cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) (Figure 1A) that support the activity of at least 90 proteins (flavoproteins) in humans.[25] Collectively, Rf, FMN, and FAD are called flavins. While Rf deficiency is rare in developed countries, suboptimal flavin status or functional deficiency can occur as a result of inadequate nutrition, age, and various health states (eg, pregnancy, alcohol abuse, digestive disorders, thyroid diseases, etc).[26] Flavins are useful for redox reactions, (as will be reviewed below), but they can also participate in other types of chemical reactions. Thus, flavoproteins are posited to serve pleiotropic roles. Flavins and flavoproteins are necessary for mitochondrial energy production,[27–29] immune system function,[30] hormone synthesis and responsiveness,[31] ROS and RNS production and detoxification, tryptophan utilization,[32–34] and neurotransmitter turnover[35,36] - among many other functions. These functions highlight the utmost importance of flavoproteins in the interaction between the immune and neural systems. Participation of flavoproteins in these broad domains suggests that alterations in flavin availability can influence neuroimmune, neuroendocrine, and metabolic activities, and consequently affect both the acute response to and long-term effects of pro-inflammatory and psychological stressors. Flavin deficiency may cause changes in affective states[37] and decrease the bioenergetic capacity for physical activity.[37–42] Notably, decreased flavin levels were found in a cohort of patients with MDD in remission.[43] Despite the link between decreased flavins and MDD, based upon the foregoing, we hypothesize that misappropriation of flavins by cells of the immune system can also have adverse mental health consequences that manifest due to increased neuroimmune signaling.

Importantly, stress and inflammation modify flavin and flavoprotein status. Stress- and inflammation-induced neuroendocrine and immune programs alter extracellular flavin availability[44–46] and intracellular allocation, as well as the level and activity of specific flavoproteins.[47,48] Indeed, under such deviations from homeostasis, with respect to cell type-specific adaptations, flavin utilization, and flavoprotein activity may shift from normal metabolic functions, mitochondrial oxidative phosphorylation,[49–51] and reduction-oxidation (redox) signaling, towards enhanced ROS/RNS generation[52,53] through upregulation of specific flavoproteins or activation of mitochondrial reverse electron transport.[54] This shift could permit increased IL-1β synthesis through redox-dependent mechanisms, which bolsters the immune response to a broad spectrum of potential threats. It may also support a cascade of IL-1β-dependent processes in the central nervous system (CNS) that orchestrate a transition towards depressive psychiatric illness. Here we discuss how stress- and inflammation-induced changes in systemic flavin availability can support redox alterations that promote increased IL-1β synthesis in the brain, leading to depression symptoms. To assess the feasibility that flavins could have a role in MDD, we screened the PubMed database for articles related to flavins and stress, infection, MDD, depression, ROS, inflammation, or IL-1β. Our query was inclusive of research articles published during any year. Additionally, while it has received relatively little attention, studies have suggested that antidepressant medications that elevate synaptic serotonin and other monoamines have significant interactions with flavins and flavoproteins.[46,55,56] These interactions may account for some of their pharmacological benefits for MDD symptoms, and also suggest that Rf availability could be a determinant of treatment efficacy. We describe the literature on this subject, as well as other translational implications of flavins for MDD patients.

Introduction to Flavins and Cellular Flavin Acquisition

Since many excellent reviews on flavins and flavoproteins and their importance in human health have been published, this general introduction will be limited to information necessary for understanding the current focus, and we refer the reader to Powers (2003),[57] Lienhart et al (2013),[25] and Mosegaard et al (2020)[58] for additional background knowledge. Carbon-based life would not be possible without cofactors to facilitate enzymatic catalysis of thermodynamically-unfavorable chemical

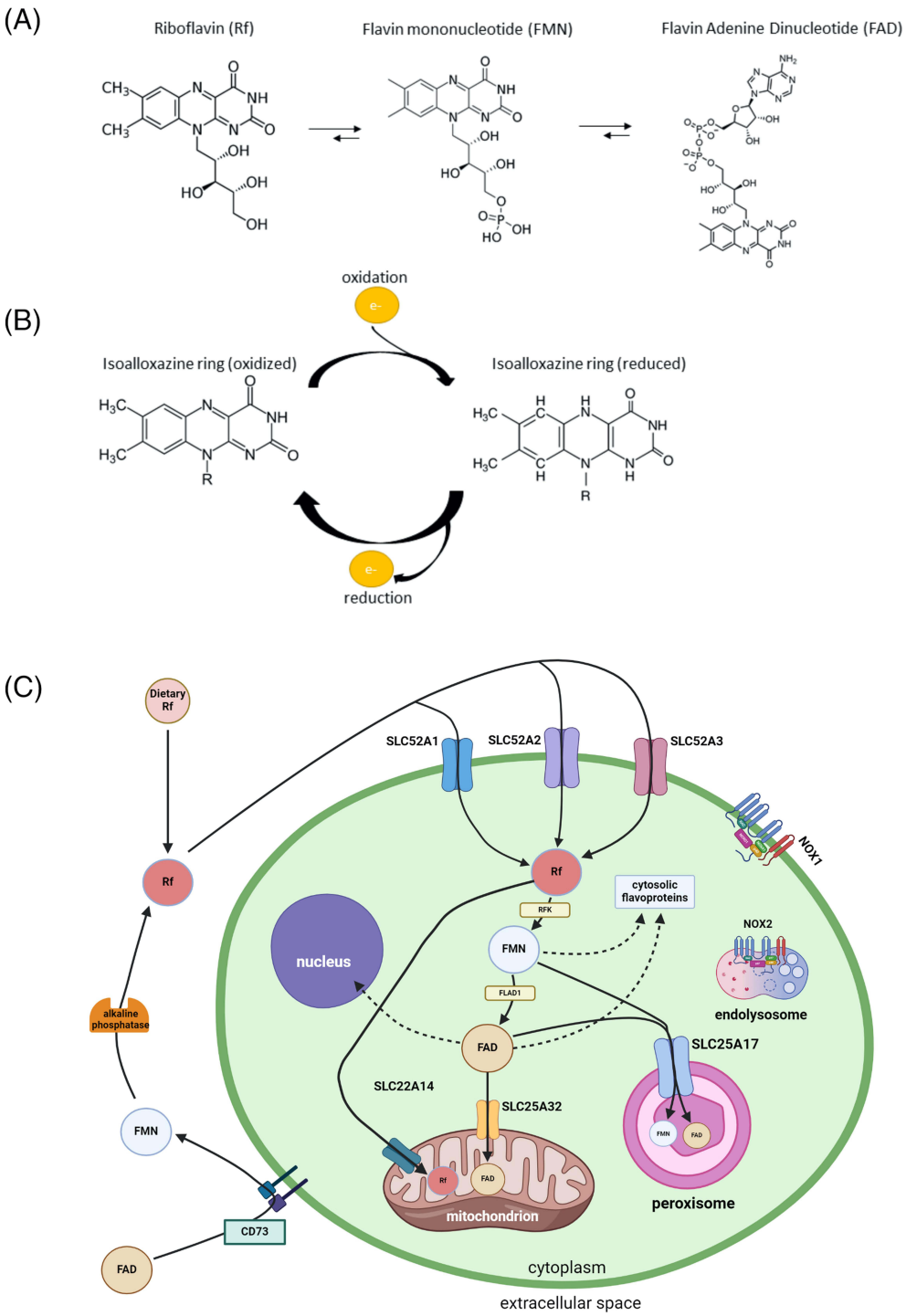


Figure 1 (**A**) Interconversion of flavins. Riboflavin (Rf) is converted enzymatically to Flavin mononucleotide (FMN) by its phosphorylation. Adenosine monophosphate is bound to the phosphate group of FMN to form Flavin adenine dinucleotide (FAD). (**B**) Basis of Flavin utility. This is a simplified schematic depicting the isoalloxazine ring participating in a redox reaction. The isoalloxazine ring can exist in three states. Nitrogen or a carbon atom of the isoalloxazine ring can accept electrons from a substrate that is then oxidized. One or two electrons may be accepted and donated. A more detailed review of the biochemistry of isoalloxazine rings can be found in Zempleni et al[170] (**C**) Cellular distribution and incorporation of flavins. Extracellular Rf is derived from diet, but it can also be regenerated from FMN or FAD by enzymatic catabolism by alkaline phosphatase and Cluster of Differentiation 73 (CD73), respectively. Cellular Rf uptake is mediated by Solute Carrier Family (SLC) 52 transporters 1, 2 and 3. Riboflavin kinase (RFK) is responsible for forming FMN from Rf. FAD synthetase (FLAD1) converts FMN to FAD. FMN and FAD are incorporated into cytosolic flavoproteins. Rf, FMN, and FAD can be distributed to subcellular compartments by a few identified transporters. SLC25A32 and SLC22A14, respectively, transport FAD and Rf into mitochondria. SLC22A14 is mostly present in a single cell type.[69] It is thought that FAD in mitochondria may be catabolized to FMN to be used for complex I.[28] Peroxisomal FMN and FAD are imported via SLC25A17. Flavins comprise many reactive oxygen species-producing enzymes in each subcellular compartment. NADPH oxidase 2 (NOX2) is localized to endolysosomes, and NOX1 is localized to the plasma membrane. Flavins and flavoproteins are also found in the nucleus. Created in BioRender. Nemeth, D (2024) BioRender.com/z17n701.

reactions. While nature has a variety of cofactors, the most versatile examples support multiple reaction mechanisms and participate in the activities of numerous enzymes, reflecting the evolution of proteins which exploit the capabilities of these cofactors. Flavins have an isoalloxazine ring system that is capable of efficiently accepting and transferring one or two electrons (and protons) via nitrogen or carbon atoms in the ring, making flavins very useful for redox-related biochemical reactions (Figure 1B). The precursor flavin, Rf, is converted to FMN and FAD (Figure 1A and B) and while FMN is used by only a handful of enzymes, FAD is used by dozens of proteins that reside in the cytoplasm and within several organelles, most notably in mitochondria. However, a few enzymes require both cofactors.

As depicted in Figure 1C, Rf is transported into cells by one of three transporters in humans: SLC52A1,[59] SLC52A2,[60,61] and SLC52A3.[62,63] At physiological concentrations, FMN and FAD cannot be taken up directly and circulating FMN and FAD must be converted back to Rf first. CD73 hydrolyzes FAD and alkaline phosphatase further degrades FMN to Rf.[64] Once in cells, Rf is phosphorylated to FMN by Rf kinase (RFK)[65] and FMN is subsequently converted to FAD by FAD synthetase (FLAD1), which is present in mitochondria and in the nucleus, where it supports nuclear flavoproteins.[66] FMN and FAD can be imported into peroxisomes by SLC25A17, which is a transporter for several other cofactors.[67] FAD can also be transported into mitochondria by SLC25A32.[68] A recently published investigation showed that SLC25A32 knockout results in less mitochondrial flavin content in cultured cells, which was due to loss of complex I of the mitochondrial electron transport chain.[28] A mitochondrial Rf transporter, SLC22A14, was characterized recently[69] that could also account for mitochondrial flavin entry, but its expression was deemed to be highly tissue-specific.

The Central Role of Flavins in Redox Biochemistry

Flavins support numerous basic cellular functions and physiological activities. FAD and/or FMN are involved in fatty acid beta-oxidation, oxidative phosphorylation, Coenzyme A synthesis,[70] Vitamin B6,[71] and B12 utilization,[72–76] circadian rhythm maintenance,[77] protein folding,[78] *reduced* glutathione regeneration,[79] tryptophan and serotonin metabolism, hydrogen sulfide metabolism,[80,81] thyroid hormone metabolism and response,[31,82–84] as well as cholesterol synthesis.[85] Most importantly for the current review, we note that flavins are crucial for their role in ROS formation and redox signaling. The vast majority of human enzymes that produce superoxide or hydrogen peroxide (H_2O_2)[86] contain flavin cofactors. Superoxide spontaneously converts to H_2O_2. However, superoxide dismutase enzymes also convert superoxide to H_2O_2. H_2O_2 then oxidizes thiol groups of vulnerable protein cysteines. The activities of many proteins that have oxidizable cysteine residues are influenced by the presence of elevated concentrations of H_2O_2. Cells use ROS-generating proteins, such as NADPH oxidases, as a means to regulate the activity of other proteins by controlling their cysteine thiol group oxidation state. The cysteine oxidation in this kind of signaling, termed redox signaling, is reversible.[87] A classic example of redox signaling is the oxidation of cysteine residues on tyrosine phosphatases by H_2O_2 following stimulation of cells with trophic factors. Oxidation of tyrosine phosphatase cysteine residues inhibits phosphatase activity, which prolongs activation of receptor tyrosine kinases (RTKs) by trophic factors.[88] RTKs induce acute NADPH oxidase activity. The superoxide produced is converted to H_2O_2, which inhibits the phosphatases. The particular importance of redox signaling in initiating and sustaining inflammation will be explained in further detail in Flavins, Flavoproteins, and IL-1β in Stress Sensitization, and MDD.

Stress, Inflammation, IL-1β Signaling, and MDD

Psychological and chronic stressors are risk factors for and often precipitate MDD episodes.[89] The primary physiological response to a stressor is the activation of the HPA axis. Exposure to stressful events increases the release of corticotropin-releasing factor (CRH) from the paraventricular nucleus of the hypothalamus, which binds to CRH receptors to induce adrenocorticotropic hormone (ACTH) release from the anterior pituitary. ACTH binds to ACTH receptors on cells of the adrenal cortex. ACTH receptors mediate secretion of glucocorticoids. Glucocorticoids induce a range of effects that may contribute to induction of depression; as reviewed elsewhere.[90] Notably, glucocorticoids exert immunosuppressive effects through binding to the intracellular glucocorticoid receptor, which suppresses transcriptional activity of the pro-inflammatory transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). The glucocorticoid receptor also suppresses inflammation through a recently described metabolic effect.[91] However, during chronic or

severe stress, glucocorticoid-resistance may occur, which antagonizes the immunosuppressive effect of the glucocorticoid receptor on NF-κB.[92] One cause of glucocorticoid-resistance is downregulation of glucocorticoid receptor protein levels.[93]

Glucocorticoid-resistance leads to increased immune activation; both of which are commonly documented occurrences in MDD subjects.[94] A correlate of immune cell reactivity is the production of cytokines and increased peripheral levels of pro-inflammatory cytokines have been detected in many MDD studies.[95] Some of those studies found IL-1β to be increased. The inconsistency of changes in peripheral IL-1β may be explained by the typically short duration that IL-1β is elevated during an immune response[96] and by the inaccuracy of measuring minor changes in IL-1β protein, which exist at extremely low (sub-picogram per 100 ug protein in the brain under basal conditions) abundance.[96] Several clinical studies also detected increased expression of inflammasome components in MDD subjects.[97–99] These results are consistent with findings of increased inflammasome expression and/or IL-1β production in animals following in vivo stress paradigms,[93,100–102] selective upregulation of NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) expression in the brain of stress-susceptible but not stress-resilient mice,[103] and a requirement for NLRP3 expression for stress sensitization.[104] It should be noted that the involvement of IL-1β in mediating many of the outcomes of stress requires its interaction with neuronal IL-1R1, although studies have shown that blood-brain barrier endothelial cell IL-1R1 expression is also important for stress pathology.[100,102]

In the CNS, pro-inflammatory elevations in IL-1β are largely due to stimulation of microglia,[18,105,106] and microglial depletion has revealed their importance for the observed behavioral effects of stress.[100,107] Following stress, microglial glucocorticoid-resistance occurs and causes increased microglial IL-1β production.[92,93] Our lab, along with collaborators, have shown that hippocampal dentate gyrus glutamatergic neuronal IL-1R1 expression is requisite for neuronal adaptations to stress, as well as accompanying cognitive deficits and behavioral responses.[108] Mice lacking neuronal IL-1R1 (nIL-1R-null mice) are less prone to developing social deficits and anxiety following social defeat stress. The precise downstream mechanisms of glutamatergic neuronal IL-1R1 activation that mediate stress sensitization still need to be elucidated. However, following a multi-day social defeat stress paradigm, nIL-1R1-null mice do not display increases in the neuronal activation markers Delta FosB or phospho-CREB,[108] implying that neuronal IL-1R1 is required to encode the stressful event.

Additional aspects of IL-1β/IL-1R1 signaling may contribute to depression. Exposure to sufficient levels of cytokines or to toll-like receptor agonists, such as lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid (Poly (I:C)) induces a set of behaviors known as sickness behaviors, which include fatigue, decreased locomotor activity, and decreased sociability, fever, and decreased food consumption. Some sickness behaviors are like behaviors seen in humans during depression and it has been suggested depressive features could arise from the same neural pathways that mediate some sickness behaviors.[109] Our lab showed that sickness behavior induced by intracerebroventricular injection of IL-1β was dependent on brain endothelial cell IL-1R1 and required the expression of endothelial cyclooxygenase-2.[18] In another study, peripheral administration of low-dose (0.2 mg/kg) LPS led to distinct depression-like behaviors in mice within one hour without the symptoms of a typical sickness response, but this did not occur in IL-1R1-knockout or in serotonin transporter- (SERT) knockout animals.[15] It is believed that the depressive-like effects of LPS observed by Zhu et al[15] were induced by increased activation of SERT by IL-1R1 expressed on serotonergic neurons. Serotonergic neuronal IL-1R1 increases SERT activity through activation of p38α MAPK.[24,110] Such behavioral responses to low-dose LPS did not occur in mice that had serotonergic neuron-specific genetic ablation of p38α MAPK.[111] Other effects of increased CNS IL-1β with relevance for depression include the ability of IL-1β to impair hippocampal neurogenesis,[112] antagonize brain-derived neurotrophic factor signaling,[113,114] promote kynurenine pathway activation,[112] and stimulate the HPA axis.[115,116]

Flavins, Flavoproteins, and IL-1β in Stress Sensitization, and MDD

Stress-Induced Changes in Flavin Levels

An important function of glucocorticoids is to increase nutrient availability to assist the body to adapt to stressors. Both psychological stress and pathogen infection,[45,46,117] a physiological stressor, temporarily increase circulating flavin levels via mobilization from hepatic tissue. This may be due to elevated blood glucocorticoids.[118–120] Under glucocorticoid exposure, the liver may release other B vitamins, in addition to flavins.[119] Transient elevation of plasma B vitamins and

their mature cofactors, including FAD, was also observed in mice injected with ATP,[121] which highlights the generalizability of this biological response to stressors. Brijlal et al (1996)[117] suggested that the increase in flavin mobilization during infection may support the immune response against pathogens. FAD released by the liver during inflammation is broken down extracellularly to FMN and Rf. Consequentially, an enhanced provision of Rf becomes available to peripheral leukocytes during periods of elevated exposure to stressors. Following uptake by leukocytes, intracellular Rf is converted back to FMN by RFK and subsequently to FAD, which are incorporated into flavoproteins involved in pathogen defense (eg, inducible NOS; iNOS).

Specific Roles of Flavoproteins in Neuroinflammation and MDD

Similar to the conceptualization proposed by Brijlal et al,[117] we postulate that during psychological stress-induced HPA axis activation, acute flavin redistribution from the liver compartment to the bloodstream functions to enhance both peripheral- and CNS immune activation, contributing to pathological immune-related processes that enhance stress susceptibility (Figure 2); these activities may include ROS synthesis, increased kynurenine pathway metabolism, and cytokine production. In support of this idea, corticosterone treatment increased plasma Rf in a rat postpartum depression model, and the length of time that rats spent immobile in the forced swim test was positively correlated with plasma FMN.[120] Corticosterone concurrently increased 3-hydroxykynurenine, the breakdown product of kynurenine produced by the flavoprotein kynurenine-3-monooxygenase (KMO). 3-hydroxykynurenine is a toxic metabolite that induces ROS production.[122,123] The authors of a social-defeat stress study,[46] assigned C57BL/6 mice into stress-susceptible- and stress-resilient groups based upon their willingness to interact with a novel CD-1 mouse after 10 days of undergoing social-defeat stress. Metabolomic data was acquired from stress-susceptible, stress-resilient, and control mice. The authors observed that mice which were susceptible to social-defeat stress tended to have higher serum FAD Z-scores than both control mice and stress-resilient mice. Lastly, serum levels of the ROS-producing flavoprotein NADPH oxidase 1

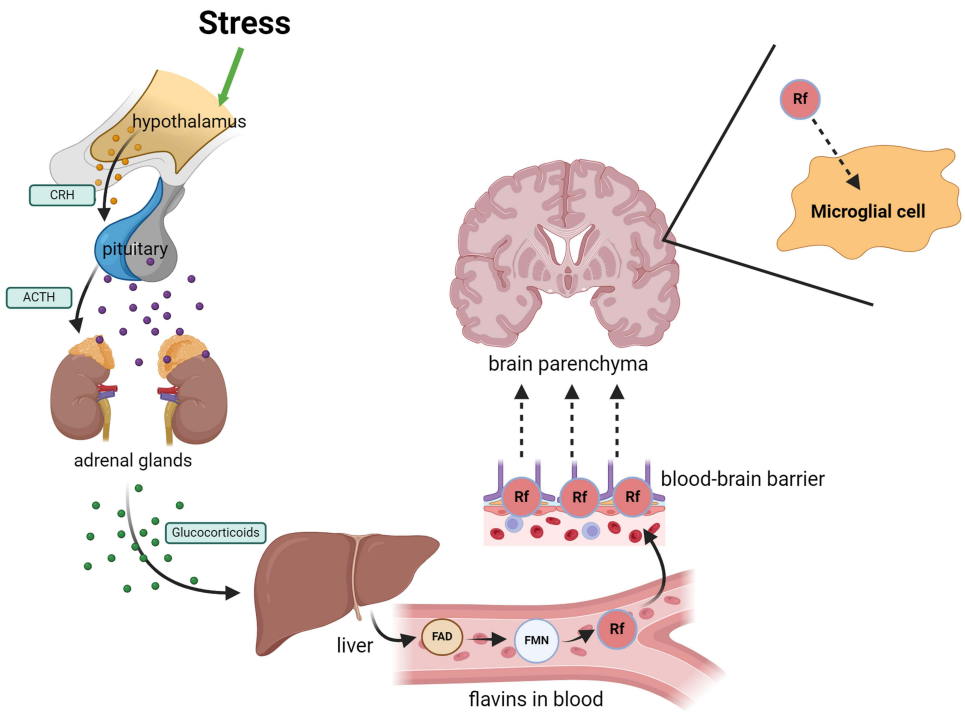


Figure 2 Paradigm for increased nutrient availability to microglia during stress. Psychological stress induces a cascade of events that may culminate with increased riboflavin (Rf) availability to cells of the brain parenchyma. A microglial cell, the main focus of this text, is depicted as an example. The proposed increase in Rf for microglial utilization may be important for the development of depressive phenotypes. Not shown here, is the activation of the hypothalamic-pituitary-adrenal axis by cytokines in the hypothalamus or pituitary. A cytokine-dependent elevation in glucocorticoids would presumably initiate the same release of hepatic Rf. Adrenocorticotropic hormone (ACTH); Corticotropin-releasing hormone (CRH); Flavin adenine dinucleotide (FAD); Flavin mononucleotide (FMN); Riboflavin (Rf). Created in BioRender. Nemeth, D (2024) BioRender.com/t06q366.

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(NOX1) were increased in patients with MDD and were positively correlated with Hamilton Rating Scale for Depression (HAM-D) scores.[124] The finding of increased NOX1 is consistent with the conclusion of a meta-analysis that found increased ROS in MDD.[125] When taken together, the studies described above suggest that blood flavin levels are specifically increased in stress-susceptible animals. In turn, increased flavins and stress susceptibility appear to be linked with increases in the level or activity of flavoproteins expressed in immune cells that produce ROS.

How might these factors, namely flavins, redox status, and IL-1β, be related and do they have a causal influence on stress sensitivity and depression? Tight connections between inflammation and cellular redox status are well-known, with studies demonstrating that ROS at the proper concentration typically induce molecular changes that are supportive of pro-inflammatory activities.[126] Since flavins are cofactors for ROS-generating enzymes, an increase in circulating flavins during states of psychological stress may heighten stress sensitivity through redox-based facilitation of cytokine (especially IL-1β) generation. In turn, elevated cytokines modulate neural activity in brain regions that are involved in depression. In leukocytes, flavoprotein-dependent ROS (generally H_2O_2) formation causes oxidation of redox-sensitive cysteine residues in proteins or protein complexes that, when oxidized, may promote IL-1β transcription, maturation, or release (Figure 3).

Specifically, ROS stimulate NF-κB-dependent transcription of several cytokines, including IL1b, and chemokines. While several studies show that ROS generation by NADPH oxidases[127,128] and other flavoproteins, including xanthine oxidase/dehydrogenase (XDH)[129,130] and monoamine oxidase b (MAO-B),[131] are permissive towards IL-1β production, there is mixed evidence for the importance of ROS in aiding the conversion of pro-IL-1β to mature IL-1β via NLRP3 inflammasomes.[132] Should ROS production by flavoproteins not prove to be responsible for promoting inflammasome oligomerization or function, other redox-related mechanisms of flavoproteins may be relevant, including oxidative phosphorylation-dependent creatine synthesis.[133] Mature IL-1β release is enhanced by ROS.[134] It is known that cysteine residues in gasdermin D are sensitive to modifications.[135–137] ROS are important for palmitoylation of gasdermin D cysteine residue 191 and this is essential for channel formation.[136]

The importance of Rf availability as an IL-1β-regulating factor was shown in a study by Mazur-Bialy et al (2015),[138] who found that lipopolysaccharide- (LPS) stimulated RAW 264.7 macrophage cells cultured in media with an Rf concentration of 3.1 nM, (representative of moderate Rf deficiency), secreted less IL-1β than cells cultured in media containing 10.4 nM Rf. Additional evidence comes from studies demonstrating that flavins, which are more available to leukocytes during stress due to increased mobilization from hepatic tissue, serve as cofactors for ROS-producing enzymes needed for IL-1β transcription, maturation, release, and IL-1β/IL-1R1 signaling; moreover, canonical signaling downstream of IL-1R1 requires NADPH oxidase activity and ROS formation.[127,128]

Increased peripheral cytokines transduce signals to the CNS. The above-described mechanisms likely occur in a parallel manner within the brain parenchyma, thereby contributing to immune-mediated neuronal stress sensitization on two fronts. During stress, in addition to being taken up by peripheral leukocytes, the excess Rf released into blood likely crosses the BBB endothelium (Figure 2). Transcriptomic analysis of brain tissue from C57BL/6 mice subjected to a social-defeat stress paradigm,[108] detected elevated hippocampal mRNA expression of the Rf transporter SLC52A3. Single-cell sequencing data[139] indicates that hippocampal SLC52A3 is almost exclusively expressed by endothelial cells. Therefore, it is possible that stress enhances Rf transport into the brain parenchyma. Extracellular Rf in the brain parenchyma is available for import into various cell types, including the innate immune cells of the CNS, microglia.

Increased levels of cytokines in the periphery lead to the transmission of inflammatory signals to the CNS via afferent pathways, causing microglial reactivity. Reactive microglia produce more IL-1β, and microglial to neuronal IL-1β/IL-1R1 signaling can have a variety of depression-related effects that are mediated by specific populations of neurons, as described in the Introduction. Although IL-1β maturation and release by microglia may be less dependent on caspase-1 activity than IL-1β production by peripheral leukocytes,[140] increased incorporation of Rf into microglial flavoproteins may enhance IL-1β maturation in microglia as well. Evidence suggests that iNOS,[141] NADPH oxidase,[142,143] KMO,[144] and XDH[145,146] are important for microglial-dependent inflammation. Microglial reactivity can also be stimulated by elevated astrocytic MAO-B.[147] Furthermore, RFK is upregulated during and supports CNS inflammation. Zhang et al (2023)[148] found that RFK is detectable through immunohistochemistry in microglia, but not in neurons or astrocytes. In their study, LPS exposure increased RFK protein in BV2 microglial cells, as well as in mouse cortex and hippocampus.

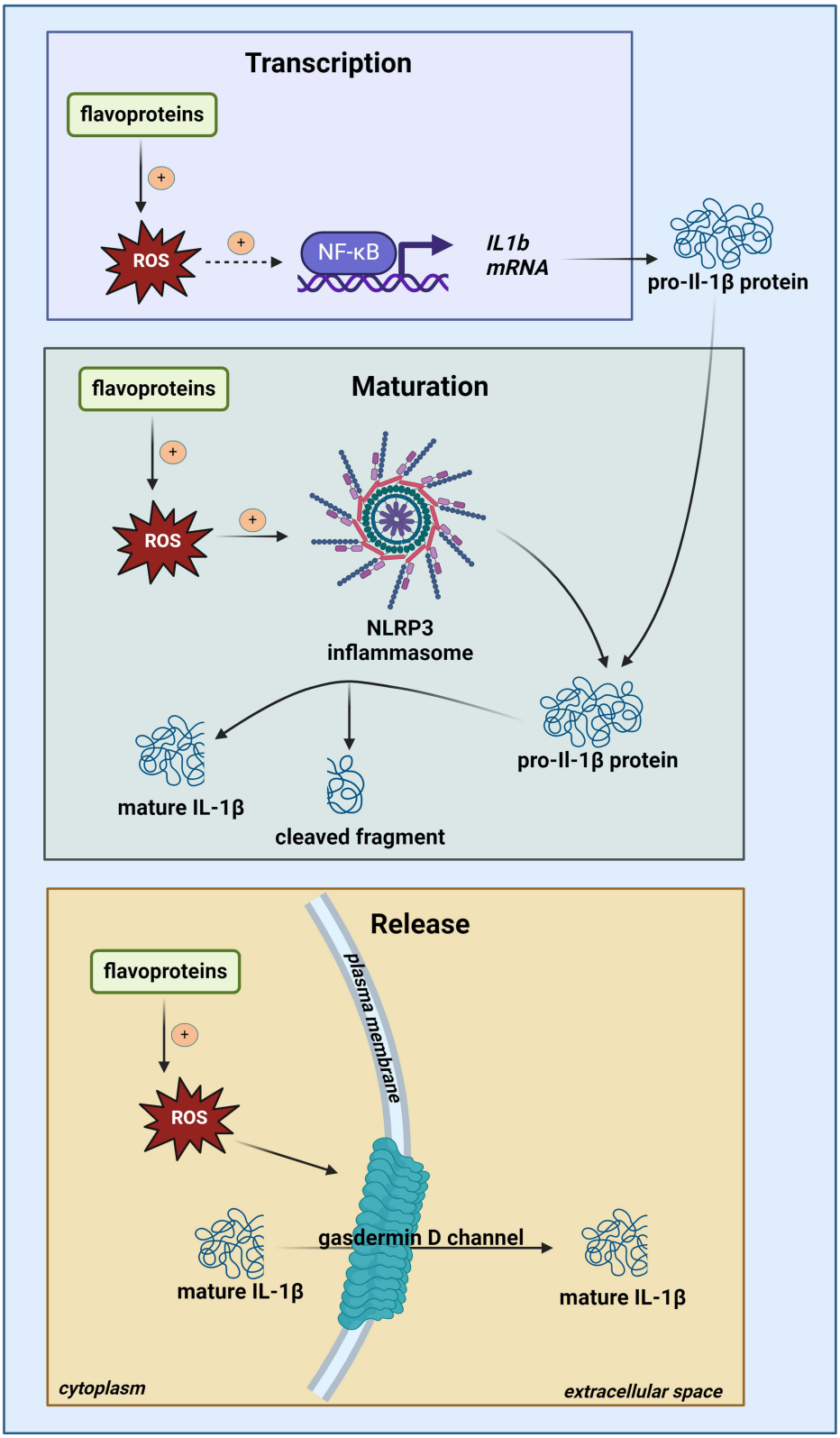

Figure 3 Flavoprotein-dependent reactive oxygen species production is essential for proper IL-1β expression by immune cells. The majority of enzymes that produce the reactive oxygen species (ROS) superoxide and hydrogen peroxide are flavoproteins. Significantly higher than ambient ROS levels enhances transcription by nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) through redox modification of upstream signaling regulators. Of these transcribed genes, IL1b mRNA is translated to pro-IL-1β protein, which is cleaved to a less massive IL-1β protein. This is typically achieved by caspase 1 enzymes associated with NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasomes. The role of ROS in pro-IL-1β processing to its mature form by NLRP3 inflammasomes is unclear.[132] However, evidence has come to light in that ROS are necessary for the release of IL-1β.[135] Release of mature IL-1β to the extracellular space is required for its signaling. Created in BioRender. Nemeth, D (2024) BioRender.com/j94p705.

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Furthermore, siRNA-mediated knockdown of RFK in both BV2 cells and in primary microglia showed that RFK was important for several pro-inflammatory effects of LPS. The ability of RFK siRNA to attenuate the effects of LPS was especially pronounced in primary microglia, where a decrease in IL-1β mRNA was detected upon RFK knockdown. Knockdown also blocked an increase in secreted IL-1β protein in BV2 culture supernatant, thus highlighting that microglial IL-1β production is sensitive to RFK status.

Translational Implications

Biomarker Prospects of Flavins

While flavins may play a role in pathological neuroimmune processes through their function as cofactors in ROS and nitric oxide production, flavins lack specificity to serve as diagnostic biomarkers for MDD. This is because, as noted in earlier in this manuscript, a collection of insults (including infection, inflammation, psychological stress, and other biological stressors) can cause a transient increase in circulating flavins. Another issue is that flavin levels can actually be decreased in MDD patients.[43,149] Infection[44,45,117] and elevated tumor necrosis factor-alpha[150] can, respectively, stimulate urinary flavin excretion and decrease intestinal Rf uptake. Chronic glucocorticoid[119] exposure also decreases circulating levels. Therefore, over an extended period of time under stressful conditions, it would be expected that circulating flavin levels will decrease relative to levels found in healthy states. Decreased circulating or solid tissue levels of one or more flavin, or biochemical tests of erythrocyte FAD status (an increased erythrocyte glutathione reductase activation coefficient), have not only been reported in MDD patients, but have also been observed in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome,[151] Long COVID,[171] HIV,[152] Crohn's Disease or Ulcerative Colitis,[153,154] and chronic obstructive pulmonary disease.[155] However, plasma FAD was decreased shortly (around 2 days) after elective knee surgery and rebounded to pre-surgical levels by one week post-surgery,[156] representing an exception to decreases after prolonged stressors. These findings as a whole imply that changes in flavin abundance may be a common finding across multiple disease states and cannot generally help to distinguish between illnesses or health conditions. As discussed by Berk (2023)[157] there are numerous types of biomarkers that are clinically-relevant, and it is possible for flavins to serve as biomarkers for purposes unrelated to MDD diagnosis. For example, specific levels or changes in flavins in responders to therapies can be explored for predictive potential. In a small recent study, FMN levels at baseline were correlated with improvements in depression symptoms after intermittent theta burst stimulation on the dorsolateral prefrontal cortex.[172]

Genetic variants in flavin acquisition and/or processing genes could also potentially serve as biomarkers for therapeutic efficacy. In addition to contributing to stress sensitization (Figure 4), it is possible that flavins, RFK, and ROS-producing flavoproteins also influence antidepressant efficacy. The involvement of these factors in microglial redox-dependent IL-1β signaling to neurons offers a plausible explanation for the findings of Ji et al (2013).[158] Ji et al performed a genome-wide association study to assess genetic factors associated with clinical responsiveness to the SSRIs citalopram and escitalopram in MDD. No genetic variants were significant following false-discovery rate correction. However, a single nucleotide polymorphism (SNP) (rs11144870) in intron 2 of the RFK gene was the most significantly associated SNP detected ($P = 1.04\times10^{-6}$) and was linked with a poorer 8 week therapeutic response. The authors probed the molecular impact of this SNP by introducing it into cultured human cell lines, where it was found to increase RFK promoter activity in a luciferase assay relative to cells without the SNP. This suggests that higher RFK transcription decreases the effectiveness of citalopram and escitalopram in MDD by increasing cellular Rf utilization. The presence of the SNP could conceivably increase microglial IL-1β production to a greater extent in SNP carriers. Higher brain IL-1β levels in SNP carriers may cause a less effective SSRI response through stronger induction of p38α MAPK phosphorylation in IL-1R1-expressing serotonin neurons. An alternative hypothesis is that the presence of the SNP strengthens neural circuits involved in rumination or other depressive symptoms, by increasing neuronal Rf usage. Overall, while the above findings are interesting, further research is needed to increase confidence for the analysis of FMN levels or RFK SNP rs11144870 to be deemed to have predictive value.

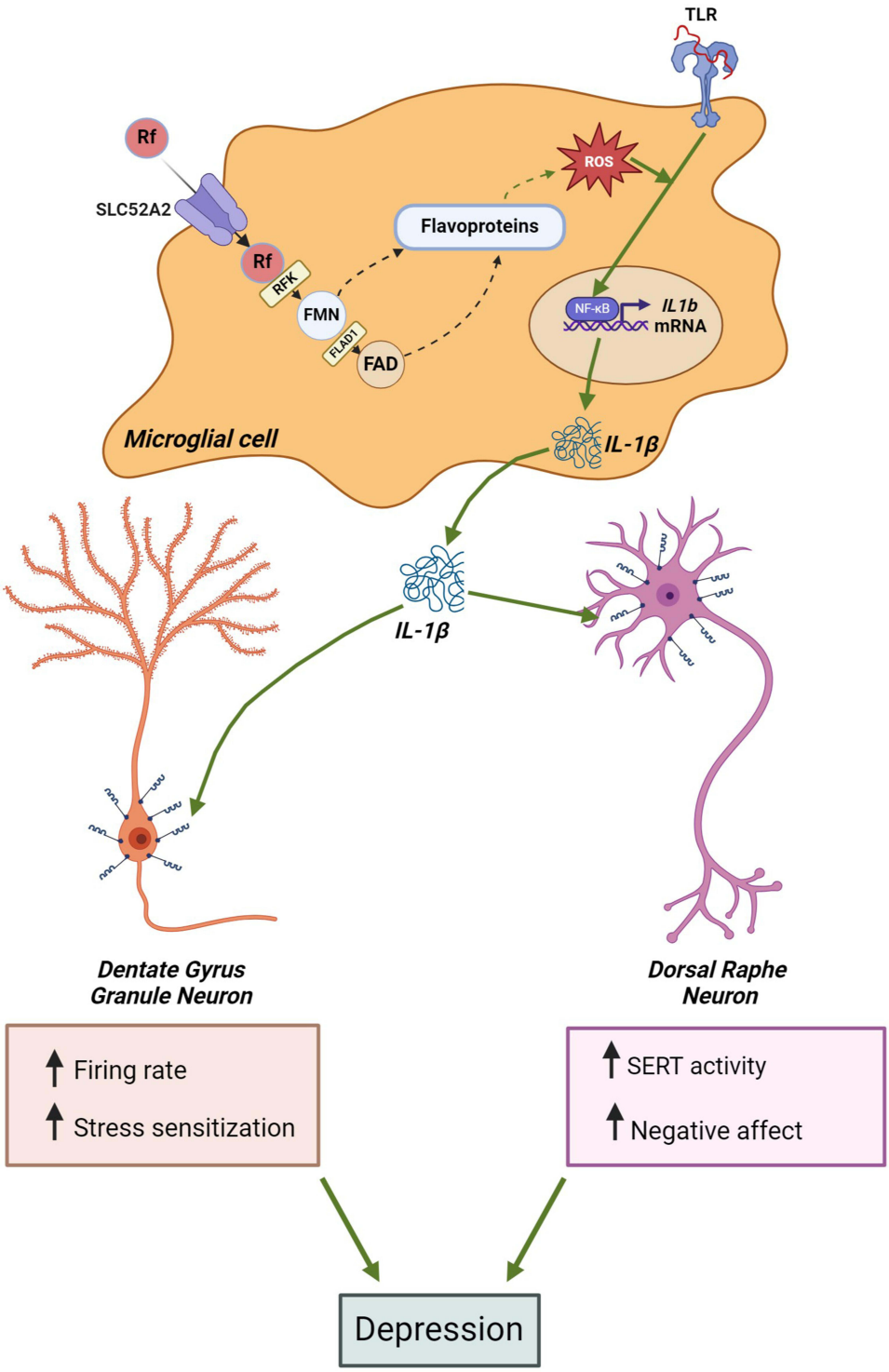

Figure 4 Riboflavin is an important factor for stress sensitization and depression via enhancement of microglial IL-1β production and neuronal IL-1R1 signaling. As discussed earlier, microglia may develop glucocorticoid resistance during periods of increased psychological stress and may contribute to stress sensitization by increasing IL-1β. Concurrently, glucocorticoids stimulate Rf release from liver storage sites and this activity may lead to increased brain parenchymal riboflavin (Rf). Microglial Rf utilization supports flavoprotein-dependent ROS formation, facilitating the transcription and potentially the processing, as well as the release of IL-1β. IL-1R1 is mainly expressed by glutamatergic neurons of the dentate gyrus, as well as serotonergic neurons of the dorsal raphe nucleus. Stimulation of IL-1R1 on these neurons leads to differential effects that contribute to depression. Dentate gyrus glutamatergic neuronal IL-1R1 stimulation causes stress sensitization by supporting the encoding of representations of stressful experiences, whereas activation of IL-1R1 on serotonergic dorsal raphe neurons decreases extracellular serotonin. Diminished extracellular serotonin can induce a negative affect. Toll-like receptor (TLR). Created in BioRender. Nemeth, D (2024) BioRender.com/i83f212.

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Pharmacological Strategies

Drugs that lower Rf availability to microglia and leukocytes have the potential to mitigate stress-induced IL-1β signaling. Intriguingly, tricyclic antidepressants (TCAs) (eg, imipramine) have been found to decrease flavin content and Rf conversion to active flavins in the brain and in other tissues.[46,55] This action has been proposed to be due to the structural similarity between Rf and TCAs. Hamilton et al (2020)[46] found that imipramine treatment decreased serum FAD and ventral hippocampus Rf in stress-susceptible mice and alleviated behavioral deficits. Lowering flavin availability to cells of the immune system could be an unappreciated anti-inflammatory mechanism of TCAs that operates in conjunction with the antidepressant effect of increasing extracellular serotonin through SERT antagonism.

Careful attention is needed for considering pharmacological strategies to help MDD patients, as treatment of MDD patients with broad irreversible inhibitors of flavoproteins, such as diphenyleneiodonium, could result in unintended systemic effects. Targeting specific flavoproteins involved in ROS generation is a more preferable strategy. Inhibitors of specific flavoproteins that can reduce ROS and inflammation are already used clinically for MDD (eg, the MAO inhibitor selegiline) and other disorders, including gout (eg, the XDH inhibitor febuxostat). MAO inhibitors can diminish ROS production and IL-1β release by peripheral leukocytes, but the main expressers of MAOs in the CNS are astrocytes[35] and neurons.[159] MAO inhibitors would thus not be expected to alter microglial IL-1β release due to suppression of microglial ROS production, but could dampen microglial inflammatory processes supported by elevated astrocytic MAO-B. Since XDH is expressed by microglia,[145] an XDH inhibitor could be a strategy to explore for depression. Allopurinol and febuxostat decreased immobility time of Swiss Albino mice in the forced swim test,[160] although the mice in that study did not undergo a stress paradigm.

Another approach that could be exploited for inflammation-related depression is the design of drugs that antagonize the physical interaction between RFK and specific client flavoproteins expressed by microglia. This class of drug was proposed by Shan et al (2024)[161] as an anti-inflammatory strategy. RFK is known to engage in physical interactions with other proteins to stimulate ROS and nitric oxide formation.[161–165] Therefore, such drugs could also prove useful for impairing ROS-dependent IL-1β release. These RFK-interaction antagonists would also need to be tested to ensure that they do not have the off-target effect of inhibiting flavoproteins involved in supporting cellular antioxidants (eg, glutathione reductase).

Lastly, an unexpected treatment possibility for inflammation-associated depression may come in the form of supplementation with high doses of flavins. Flavin supplementation has been carried out in both pre-clinical and clinical trials, where it has mostly demonstrated anti-inflammatory effects (Supplementary Table 1). Intriguingly, dietary intake of Rf has a negative association with depression risk.[166,167] These findings seem to contradict the hypothesis that increased flavin availability is supportive of inflammation-linked depression. One possibility is that dietary flavin availability shows a non-linear relationship with depression, which is supported by the findings of Wu et al (2023).[166] In this scenario, a severe lack of Rf can lead to molecular changes that are linked with depression, such as oxidative stress[168] and altered endocrine responsiveness; Optimal Rf intake would promote homeostatic functions; Mild to moderate increases in flavin availability may elevate inflammatory potential to permit pathological changes associated with depression under conditions of psychological stress; Finally, significantly elevated flavin availability, achieved via clinically-supervised supplementation, may attenuate inflammation. Pharmacologically elevated flavins can decrease RFK mRNA expression,[148] abrogate inflammation-induced increases in IL-1β mRNA and protein,[148] and inhibit NLRP3 inflammasome-mediated processes by interfering with caspase 1 activity.[169]

Conclusion

The intricate interplay between flavins, flavoproteins, and IL-1β/IL-1R1 signaling within the neuroimmune landscape underscores their pivotal roles in stress sensitization and MDD. Increased HPA axis activity downstream of psychological stress alters flavin availability, influencing redox-based mechanisms within leukocytes and microglial cells that, respectively, contribute to elevated peripheral and CNS cytokine production. IL-1β production is specifically influenced by these changes, due to the potential redox sensitivity of the NLRP3 inflammasome and gasdermin D. IL-1β/IL-1R1 signaling is a central factor in promoting stress sensitization, depression-like sickness behavior, and decreased serotonin availability. Via their

support of several ROS-producing flavoproteins, flavins serve as a metabolic bridge linking psychological stress, redox alterations, immune system reactivity, IL-1β/IL-1R1-dependent neuronal modulation, and depression. Contrary to what may occur during the development of depressive conditions, levels of certain flavins may be decreased in MDD. This can be explained by the influence of cytokines and glucocorticoids on flavin absorption and urinary excretion.

A number of health conditions are mentioned in the Biomarker Prospects of Flavins subsection that are linked with decreased flavins. A key limitation to our proposed mechanism on the contribution of flavins to stress sensitization and MDD development is that it does not account for how flavin levels and cellular utilization may vary based on lifestyle factors and comorbid conditions. While we have touched upon the relevance of nutrition, we cannot state with certainty how lifestyle factors, such as alcohol or drug abuse, or comorbid conditions, (eg, autoimmune disease), will influence the applicability of the concepts discussed in this review. Based upon there being decreased flavin levels in several conditions, as well as the literature we outlined in the preceding sections, the directionality of changes in flavins appears to be temporally influenced, with short-term stressors increasing flavins and long-term stressors decreasing flavins. These aspects make flavins a poor diagnostic marker of any particular condition. There is a possibility that flavin levels could become biomarkers predicting or answering other questions important for MDD treatment.

The suggested association between genetic variation in the RFK gene and citalopram responsiveness highlights the potential for identifying treatment responders based on personalized screening of genes involved in flavin metabolism to enhance therapeutic outcomes. However, justification for clinical implementation of such a screen requires further validation in a larger study cohort in order to obtain a level of significance greater than the false-discovery rate threshold. Another finding that has been replicated in multiple studies is the antagonistic effect of TCAs on tissue flavins. It is currently unclear if any of the antidepressant effects of imipramine and other TCAs are related to flavin-lowering effects. Experiments on SERT-mutants insensitive to the antidepressant effects of TCAs, similar to the concept of SERT Met172 mice,[173] could help to address this question.

We recommend investigating drugs that inhibit ROS- and nitric oxide-producing flavoproteins or their interaction with flavin processing proteins,[163] as well as studies examining flavin supplementation for depression. Understanding the nuanced involvement of flavins and flavoproteins in neuroimmune processes not only illuminates the pathophysiology of MDD but also offers novel avenues for promising therapeutics aimed at alleviating stress-induced depressive states. We hope that the current review inspires additional research into these areas.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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