ENDOCRINE
SOCIETY

OXFORD

Endocannabinoid System in the Neuroendocrine Response to Lipopolysaccharide-induced Immune Challenge

Andrea De Laurentiis,[1,2,3] Fernando Correa,[2,3] and Javier Fernandez Solari[1,3,] (ID)

[1]Universidad de Buenos Aires (UBA), Facultad de Odontología, Cátedra de Fisiología, Buenos Aires, Argentina
[2]Centro de Estudios Farmacológicos y Botánicos, Universidad de Buenos Aires-Consejo Nacional de Investigaciones Científicas y Técnicas (UBA/CONICET), Buenos Aires, Argentina; and
[3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

Correspondence: Javier Fernández-Solari, PhD, Cátedra de Fisiología, Facultad de Odontología (Universidad de Buenos Aires), Marcelo T. de Alvear 2142, C1122AAH, Ciudad Autónoma de Buenos Aires, Argentina. Email: javierfsolari@yahoo.com.ar.

Abstract

The endocannabinoid system plays a key role in the intersection of the nervous, endocrine, and immune systems, regulating not only their functions but also how they interplay with each other. Endogenous ligands, named endocannabinoids, are produced "on demand" to finely regulate the synthesis and secretion of hormones and neurotransmitters, as well as to regulate the production of cytokines and other proinflammatory mediators. It is well known that immune challenges, such as exposure to lipopolysaccharide, the main component of the Gram-negative bacteria cell wall, disrupt not only the hypothalamic–pituitary–adrenal axis but also affects other endocrine systems such as the hypothalamic–pituitary–gonadal axis and the release of oxytocin from the neurohypophysis. Here we explore which actors and molecular mechanisms are involved in these processes.

Key Words: endocannabinoid system, neuroimmunenendocrine axis, hypothalamus, oxytocin, hypothalamic–pituitary–gonadal axis

Abbreviations: AEA, anandamide; CNS, central nervous system; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; GABA, gamma-aminobutyric acid; GnRH, gonadotropin-releasing hormone; GPCR, G protein–coupled membrane receptor; HPG, hypothalamic–pituitary–gonadal; HPT, hypothalamic–pituitary–testicular; IL, interleukin; LH, luteinizing hormone; LPS, lipopolysaccharide; OXT, oxytocin; OXTR, oxytocin receptor; PVN, paraventricular nucleus; RFRP-3, RF amide-related peptide 3; SON, supraoptic nucleus; TNF, tumor necrosis factor; TRPV1, transient receptor potential vanilloid 1; VP, vasopressin.

The endocannabinoid system (ECS) is a complex intercellular signaling network that modulates a broad spectrum of pathophysiological processes. Components of the ECS are found throughout the body, particularly in the central nervous system (CNS) [1, 2] and in cells of the immune and the reproductive systems, among others [3, 4]. The ECS is composed of endogenous ligands called endocannabinoids, such as *N*-arachidonoylethanolamine (anandamide, AEA) [5] and 2-arachidonoylglycerol; a series of enzymes for their synthesis and degradation; and G protein–coupled membrane receptors (GPCR), with CB1 and CB2 being the main cannabinoid-specific receptors [5, 6]. Both the CB1 and CB2 receptors are members of the GPCR family and are coupled to pertussis toxin–sensitive Gi/o protein. Their activation suppresses adenylate cyclase and the formation of cyclic adenosine monophosphate. Only CB1 has been reported to activate other G proteins in a ligand-dependent manner in certain cells [2]. For example, CB1 expressed in astrocytes is coupled to Gq/11 and increases intracellular Ca²⁺ concentrations. Moreover, the CB1 receptor modulates the activity of several types of ion channels such as N-type, P/Q-type, and R-type Ca²⁺ channels and G protein–coupled inwardly rectifying K⁺ channels. Stimulation of CB1 leads to the activation of mitogen-activated protein kinase signaling pathways, including extracellular signal-regulated kinase 1/2, c-Jun N-terminal kinase, and p38 mitogen-activated protein kinase.

In addition, CB1 is able to signal in a G protein–independent manner through association with β-arrestin and activating the PI3K/Akt pathway [2].

Interestingly, endocannabinoids also interact with other unspecific receptors such as transient receptor potential vanilloid 1 (TRPV1) [7-9], serotonin receptors, GPR55, and peroxisome proliferator-activated receptors, among others [2, 10, 11]. TRPV1, also known as the capsaicin receptor, is a polymodal, nonselective cation channel expressed by all major classes of nociceptive neurons and is important for the detection of noxious stimuli. TRPV1 can be activated by a number of stimuli including heat, N-acyl amides, arachidonic acid derivatives, vanilloids, protons, and cannabinoids. Upon activation, calcium moves through the pore, enters the cell, and stimulates a series of calcium-dependent processes that ultimately lead to desensitization of the channel. Upon desensitization, the channel enters a refractory period in which it can no longer respond to further stimulation, leading to the paradoxical analgesic effect of these compounds [12].

The hydrolase *N*-acyl phosphatidylethanolamine phospholipase D and fatty acid amide hydrolase (FAAH) are the main enzymes responsible for the synthesis and degradation of AEA, respectively, while diacylglycerol lipase and monoacylglycerol lipase are the primary enzymes responsible for the synthesis and degradation for 2-arachidonoylglycerol [6]. Although cannabinoid receptors are present in different types of cells

and tissues, CB1 is highly located on presynaptic nerve terminals of neurons in the brain, whereas CB2 is mainly expressed in immune and bone marrow–derived cells [13]. The complexity increases since the ECS is now being expanded with several ligands that do not show an affinity for typical cannabinoid receptors but display cannabimimetic activity. True endocannabinoids are produced only "on demand" and play a crucial regulatory role in metabolic processes, behavior, reproduction, and immunity [6]. Furthermore, a proper interplay between all these compounds and enzymes, as well as their molecular targets gives the endocannabinoidome an essential role in the homeostatic response to noxious stimuli [14, 15].

Certain pathophysiological conditions such as inflammatory diseases, endotoxemia, and stress constitute immune challenges that lead to alterations in the synthesis and normal release of neuroendocrine factors. Lipopolysaccharide (LPS), an endotoxin from the membranes of Gram-negative bacteria, is a potent inducer of proinflammatory cytokine, prostaglandin, and catecholamine release, thus it is widely used to induce immune challenge, which in turn disrupts neuroendocrine systems. During systemic infections, proinflammatory cytokines reach the CNS in response to peripheral signals, arriving from peripheral sources crossing the damaged blood–brain barrier or through fenestrated capillaries located in certain zones of the brain [16, 17]. In addition, there is in situ cytokine production from a variety of cell types within the brain, mainly by microglia and astrocytes but also by neurons and endothelial cells [18].

The hypothalamus is the main center that receives a variety of peripheral information being the brain area where most of the neuroendocrine factors that regulate fundamental pathophysiological activities are produced. In fact, infectious agents, antigens, and LPS challenge rapidly activate the immune system, producing interferon gamma and cytokines that are dispatched into the brain modifying the activity of the hypothalamus [19]. It is well known that the immune response activates the hypothalamic–pituitary–adrenal axis, inducing the release of corticosterone, which modulates cardiovascular, metabolic, neuronal, neuroendocrine, and immune responses [20, 21]. Finally, glucocorticoids establish a negative feedback regulating their own production and on the immune system [22, 23]. Interestingly, the accurate functioning of the hypothalamic–pituitary–adrenal axis seems to be closely related to endocannabinoid signaling [24-28].

Of particular interest is the involvement of the ECS in inherent responses against inflammation and brain processes [3, 4, 29]. Several reports have shown that infection and inflammation induce the synthesis of endocannabinoids in different organs and tissues [6], which in turn participate as modulators in the triggered neuroimmune response [30]. During various pathological conditions, such as Alzheimer's and Parkinson's disease, multiple sclerosis, stroke, amyotrophic lateral sclerosis, traumatic injury, and bacterial and viral infections of the CNS, among others, the profiles of endocannabinoids undergo significant changes associated with the inflammation-modulating, analgesic, and protective activity of these compounds (reviewed in [14]). Moreover, endocannabinoids also modulate the neuroendocrine function. This "on-demand" production of endocannabinoids is essential for the fine-tuning of neurotransmission both in basal conditions and during immune challenges controlling

the release of neuropeptides, neurotransmitters, and hormones [31]. In both cases, the ECS acts as a mediator for the communication between neurons and glial cells in order to generate the most appropriate neuroendocrine responses in each situation, and the production of endocannabinoids varies depending on the response required.

Immune Challenge on the Hypothalamic–Pituitary–Gonadal Axis

Reproductive physiology is controlled mainly by the hypothalamic–pituitary–gonadal (HPG) axis. In both sexes, gonadotropin-releasing hormone (GnRH) is released from the hypothalamic preoptic nucleus to the anterior pituitary, which induces the release of luteinizing hormone (LH) and follicle-stimulating hormone to the circulatory system. LH is responsible for stimulating the secretion of sex steroids from the gonads, whereas follicle-stimulating hormone is the primary gametogenic hormone. The function of GnRH neurons is controlled by gonadal steroids participating in feedback mechanisms but it is also influenced by a large number of neurotransmitters, including mainly β-endorphin, dopamine, gamma-aminobutyric acid (GABA), histamine, glutamate, serotonin, kisspeptin, and RF amide-related peptide 3 (RFRP-3) in mammals. Kisspeptin is a major activator of GnRH neuron excitability, whereas RFRP-3 exerts inhibitory effects on these neurons. In fact, the mammalian RFRP-3 has been shown to be the ortholog of the avian gonadotropin-inhibitory hormone [32, 33].

It is well known that the activity of the hypothalamic–pituitary axis is under the influence of circulating sex steroids and several differences in the ECS have been registered between sexes. In fact, studies focusing on GnRH modulation by the endocannabinoids have shown different results when comparing male and female rats. These results indicate that changes in estrogen function can influence central endocannabinoid signaling [34]. It has been reported that female hormones that control reproduction are stimulated by cannabinoids, whereas the effect is inhibitory in males [35, 36]. In this sense, reports from our group showed that AEA inhibits GnRH release in ovariectomized female and male rats [37, 38].

As we previously mentioned, regarding reciprocal relationships between the HPG axis and the immune system, a mutual alteration is generated in the functioning of both systems [39, 40]. Systemic infectious processes cause the synthesis of proinflammatory cytokines in the hypothalamic medial preoptic area, where cell bodies of GnRH neurons are located [41]. Such cytokines act either directly affecting GnRH secretion or by modulating the secretion of neurotransmitters from different presynaptic neurons to those of GnRH [41-43]. On the other hand, the presence of testosterone and estrogen receptors on immune organs and cells suggests that sex hormones can influence the immune system directly. Furthermore, the removal of gonads can alter immune functioning [44].

Inflammatory diseases and systemic infections are often associated with impaired reproductive function [45]. Our group demonstrates that intraperitoneal administration of LPS disrupts reproductive capability [46, 47] and it is also known that cytokine balance in the hypothalamus modulates GnRH neurons activity and therefore the HPG axis. In particular,

tumor necrosis factor (TNF)-α and interleukin (IL)-1β are known to be the most important inflammatory molecules controlling GnRH neurons activity [48-50]. Thus, LPS administration inhibits GnRH release from hypothalamic neurons mainly through the action of IL-1β and TNF-α [51].

LPS-induced immune challenge also decreases hypothalamic levels of *Kiss1* mRNA in male, female, and ovariectomized rats while increasing *Rfrp* mRNA levels [46, 51]. Since kisspeptin and RFRP act as key regulators of GnRH and gonadotropins, a decrease in the signaling of the first and an increase of the second would be responsible, at least in part, for the decrease in hypothalamic GnRH content and serum LH level observed in rats exposed to immune challenge [46].

Glutamate is an important stimulator of GnRH release. The activation of the glutamate receptor subtype N-methyl-D-aspartic acid receptor is required for GnRH secretion [52]. A study performed by our group in 2004 showed that hypothalamic extracts of the medial basal area from male rats release lower quantities of GnRH induced by N-methyl-D-aspartic acid after incubation with the endocannabinoid AEA at a physiological concentration (nanomolar order), and that this effect is prevented by AM251, a selective antagonist/inverse agonist of CB1 receptors [36]. This result supported that neuronal CB1, mainly located in axon terminals of presynaptic neurons to GnRH neurons, participates in the inhibitory control of GnRH release in males. At the present, numerous studies have reported the effects of CB1 and TRPV1 signaling on GnRH modulators such as estradiol [53], kisspeptin [54], GABA [55], and ghrelin [56], among others.

In the context of immune challenges, studies by our group have shown that LPS stimulates CB1 expression and endocannabinoid synthesis in the hypothalamus of rats [57], which suggests a possible role of the ECS in mediating the disrupting effects of systemic infections on the neuroendocrine function. Our group has previously reported that the blockade of CB1 with AM251 prevented the inhibitory effect of TNF-α on forskolin-induced GnRH release from medial basal hypothalamic extracts of male rats in vitro, suggesting the participation of CB1 in the hypothalamic–pituitary–testicular (HPT) axis disruption induced by the immune challenge [45].

We reported evidence showing that during LPS-induced immune challenge, the hypothalamic ECS participated in the inflammatory response triggered by the mock infection while reducing the production of sexual hormones [46]. This conclusion is supported by the fact that after 3 hours of immune challenge, GnRH hypothalamic content and LH serum levels, as well as hypothalamic *Kiss1* gene expression, were significantly reduced, while that of *Rfrp-3* was increased. Contrarily, when the hypothalamic CB1 was centrally blocked with AM251, all parameters returned to basal levels. On the other hand, *Tnf-alpha* and *Il1beta* hypothalamic gene expression augmented due to the immune challenge, which increased further after CB1 blockade. Thus, the results show an anti-inflammatory role of central CB1 activation on LPS-induced immune challenge, while at the same time inhibiting the HPG axis. This mechanism appears to be crucial for survival, considering the high energy–dependent activation of the adrenal and neurohypophysial axis during an immune challenge. We postulate that the ECS mediates the inhibition of the gonadal axis for the energetic prioritization of essential functions crucial for the survival of the organism undergoing

infection or inflammation. Moreover, these results suggest that hypothalamic activation of the CB1 pathway might be needed to prevent an excessive immune response [46].

On the other hand, our group reported evidence of the participation of hypothalamic TRPV1 in the HPT axis function under physiological conditions, but not after LPS-induced immune challenge [58]. Just blocking hypothalamic TRPV1 with capsazepine increased *Tnf-alpha* and *Il1beta* mRNA levels compared with control, without showing an additive effect on the already increased cytokine levels induced by LPS. The proinflammatory effects induced by TRPV1 blockade in basal conditions could be linked to the consequent inhibitory effect on the reproductive axis, evidenced by the decrease in the expression of hypothalamic *Gnrh* and *Kiss1* mRNA and serum LH and testosterone levels. Nevertheless, TRPV1 blockade did not produce any effects on the diminished reproductive parameters observed during an immune challenge [58].

The participation of TRPV1 in the control of cytokine production remains controversial, and studies in the literature show conflicting results since the action of this receptor depends on different conditions, such as the tissue studied, the identity, and concentration of cytokines at the time of receptor activation, and the moment when its response is measured [59, 60]. As previously mentioned, our results showed that the blockade of TRPV1 increased cytokine levels in the absence of any stimuli, suggesting a constitutive anti-inflammatory action for TRPV1, whose activation might be required to abolish the expression of proinflammatory mediators under physiological conditions [58]. However, TRPV1 has also been reported to be associated with a proinflammatory status [61]. Nevertheless, during an immune challenge, the activation of CB1 seems to decrease TRPV1 expression, perhaps as a response to maintaining homeostasis. These findings are in agreement with reports from our group, showing that CB1 blockade during immune challenge increases *Trpv1* mRNA expression [46]. It can be hypothesized that TRPV1 exerts an anti-inflammatory role during physiological conditions, but switches to a proinflammatory action during the immune challenge. However, CB1 signaling, increased in such conditions due to high endocannabinoid availability, could attenuate TRPV1 signaling in order to modulate the inflammatory response.

In summary, the blockade of CB1 during an immune challenge could restore reproductive function but leads to a dangerous exacerbation of the inflammatory response, whereas the blockade of TRPV1 in physiological conditions could increase basal cytokine production leading to HPT axis inhibition. Therefore, it can be concluded that hypothalamic CB1 mainly participates in the mechanisms induced by an immune challenge, controlling the inflammatory response and mediating the inhibition of the HPT axis, while hypothalamic TRPV1 might have a role in physiological conditions, maintaining cytokines at basal levels allowing the adequate function of the HPT axis (Fig. 1).

Immune Challenge on the Hypothalamic–Neurohypophysial Axis

Exposure to an immunological stimulus activates sensory and limbic structures, but stimulation of hypothalamic neurons of the paraventricular nucleus (PVN) and supraoptic nucleus (SON) is critical for activating the hypothalamic–pituitary
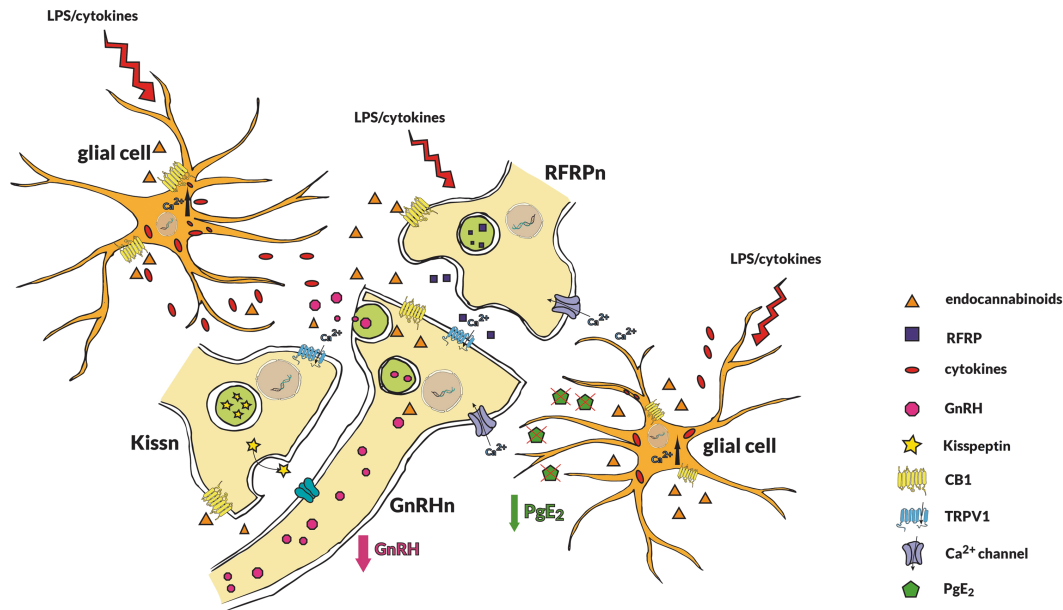
Figure 1. Hypothetical involvement of hypothalamic CB1 and TRPV1 in the HPT axis during an immune challenge. The schema contains kisspeptin and RFRP as key neurotransmitters involved in GnRHn activity and therefore in GnRH, LH, and testosterone (T) secretion, with the understanding that many other neurotransmitters must be considered. Kisspeptin stimulates GnRH production and secretion and RFRP-3 negatively modulates GnRH and kisspeptin production, as well as LH production by gonadotrophs. Under physiological conditions, CB1 signaling is attenuated due to low levels of endocannabinoids, which are maintained by TRPV1 anti-inflammatory effects. During an LPS-induced immune challenge, microglial cells and astrocytes produce TNF-α and IL-1β, which in turn induce the production of endocannabinoids. These lipid mediators not only modulate microglia actions by decreasing cytokine production but also decrease kisspeptin neurotransmission and increase RFRP. Thus, the HPT axis is inhibited as evidenced by decreased GnRH, LH, and T. Further studies are needed in order to confirm whether this hypothesis is correct or not. Kissn, GnRHn and RFRPn stand neurons for respective neurotransmitter.

axis thus triggering the neuroendocrine response to inflammation [62-64]. In this section of the review we focus on one of the hormones produced and secreted from these hypothalamic nuclei: oxytocin (OXT) [65-68]. In order to understand the complex actions of OXT, it is important to point out that this hormone plays a role not only in the brain and reproductive tissues but also in the autonomic nervous and immune systems. OXT participates in the immune homeostasis and in the resistance to immunological challenges, stressors, and insults in all tissues [69, 70]. Oxytocinergic neurons can integrate information from presynaptic neurons, sense the state of glial activation [71], detect local and blood concentrations of substances including cytokines, and secrete the appropriate amount of OXT in the brain and to the periphery, thus maintaining homeostasis of immune functions and preventing further damage [67, 72].

OXT is synthesized in the brain in hypothalamic magnocellular oxytocinergic neurons that extend their processes to the posterior pituitary where the hormone is released into the systemic circulation. These cells also produce neural projections throughout the brain and spinal cord [67]. Furthermore, OXT secreted locally in the brain can be detected within fibers that reach a considerable distance from the cells of origin [73]. The action of this hormone is exerted by its specific binding to oxytocin receptor (OXTR) with 7 transmembrane domains. The OXTR is characterized as a GPCR. It is coupled to a trimeric complex of G proteins, consisting of 1 Gα and 1 β/γ unit. Upon ligand binding, the complex separates the β/γ subunit from the Gα-protein. Several different α-protein subforms can contribute to the composition of the complex. Depending on the type of Gα protein, the functional outcome of receptor activation can be stimulatory

or inhibitory. The coupling of the receptor to the activating Gαq/11 decreases inward rectifying K⁺ currents depolarizing neurons, while coupled to the inhibitory Gα proteins leads to inhibition. OXT also induces Ca²⁺-activated K⁺ channel–mediated outward currents via the Gq/11/phospholipase C pathway. In addition to Gαq-mediated phospholipase C activation, the β/γ subunit also triggers the phosphorylation of the phospholipase Cβ3 and the OXT-induced generation of inositol-3-phosphate and diacylglycerol with the capacity to release Ca²⁺ from intracellular stores [74].

The same receptor is found in brain and peripheral tissues [74, 75]. OXTRs are located in areas of the nervous system that regulate social, emotional, and adaptive behaviors, such as the amygdala, hippocampus, hypothalamic–pituitary axis, and autonomic nervous system [76]. The binding of OXT to its receptor triggers intracellular cascades that will depend on the concentration of the neuropeptide and the amount and cellular location of the OXTR and generate effects on several transcription factors [74]. Furthermore, signaling also depends on whether or not OXTR is included in a lipid raft [77]. Lipid rafts are defined as highly dynamic, cholesterol- and sphingolipid-enriched, nanometer scale membrane domains that recruit certain proteins and are stabilized by lipid–lipid and lipid–protein interactions. For example, when OXTR is excluded from lipid rafts, OXT inhibits cell proliferation, whereas, if the receptor is located in these lipid microdomains, it can have a mitogenic effect remarking the relevance of the OXT–OXTR signaling [77-79]. All these characteristics would explain the ability of OXT to have so many functions in different tissues.

The importance of the oxytocinergic system is demonstrated by the fact that the exposure of a fetus to an inflammatory

environment during an early phase of CNS development produces a series of structural, metabolic, and epigenetic changes in this system for a longer period of postnatal life, increasing the risk of neurological and neuropsychiatric diseases, such as schizophrenia and autism spectrum disorders [80, 81]. Moreover, the oxytocinergic system is very sensitive to epigenetic modifications throughout adult life, particularly in situations of stress, infection, or trauma, revealing the great plasticity of this system [82, 83].

OXT promotes health by influencing the nervous and immune systems with its neuroprotective, anti-inflammatory, and antioxidant roles, both in childbirth and against insults in adult life. Recent findings consider OXT as a neuroprotector of the fetal brain since it prevents the damage that can occur during birth, which is a powerful inflammatory/immunological event [69, 81]. OXT protects fetal neurons from the hypoxic and inflammatory conditions of childbirth by modifying the action of neurotransmitter signaling, inhibiting microglia, and reducing oxidative stress [84]. OXT is involved in the control and protection of brain tissue in adulthood since it has been shown that OXTR increases its expression in brain regions with microvascular damage in individuals with Alzheimer's disease. This is a specific response, given that the signaling cascades of this receptor provoke antioxidant, anti-inflammatory, and proangiogenic responses [85].

Although OXT is one of the best described physiological and behavioral modulators, the pathophysiological and cellular consequences of OXT signaling are poorly understood and still present numerous contradictions and complexities. Contributing to this puzzlement is the fact that OXT integrates a system with vasopressin (VP), even though they have different signaling cascades [86]. Furthermore, precursor forms of OXT are biologically active in brain [87], and in addition, the oxytocinergic system also interacts with many components of the hypothalamic–pituitary–adrenal axis as well as with acetylcholine, GABA, glutamate, opioids, catecholamines, steroids, and endocannabinoids [74, 88].

Circulating OXT can inhibit inflammation, exert an antimicrobial effect, promote wound healing, and suppress immune disorders associated with stress. In this process, the hypothalamus can release OXT to act on the immune system directly by activating OXTR or by modulating the activity of other systems indirectly [67, 68]. To understand the role of the oxytocinergic system in regulating the immune system, our studies focus particularly on its relationship with the ECS.

The ECS is currently an important neuromodulatory system whose primary function is to maintain homeostasis [6, 9]. As stated in previous sections, the proper interplay between all the elements of the ECS is essential for the homeostatic maintenance of a number of physiological, cognitive, behavioral, and emotional processes. In fact, several studies highlight the anti-inflammatory role of the ECS [1, 89-91].

As previously mentioned all components of the ECS are present in the organs that constitute the hypothalamic–pituitary axis [92] and can control the release of hypothalamic neuropeptides and pituitary, gonadal, and adrenal hormones. The hypothalamic–neurohypophysial system is a neuroendocrine system essential for survival, and due to the remarkable role of the ECS as a prohomeostatic neuromodulator [14, 15], understanding the interplay between the 2 becomes imperative. In 1991, Herkenham et al first reported that CB receptors are localized in the PVN of the hypothalamus and in the pituitary lobe [93]. Subsequently, we determined the presence of immunoreactive neurons for the CB1-type cannabinoid receptor adjacent to the cerebral ventricle, an area with a predominance of oxytocinergic magnocellular neurons [94]. It has been reported that endocannabinoids are released as retrograde messengers by magnocellular neurons and CB1 receptors are localized within the SON, suggesting that endocannabinoids could modulate the physiology of oxytocinergic neurons [95]. Next, one report highlighted the interaction between endocannabinoids and the modulation of the physiology of magnocellular neurons, since OXT and endocannabinoids cooperate to shape the electrophysiological properties of SON neurons [96]. Two years later, we demonstrated the direct influence of the ECS on the production and release of OXT and the intimate relationship between nitric oxide (NO) and the hypothalamic oxytocinergic and ECS, which provides regulation of the systemic inflammatory and stress responses [57, 89, 90]. Our in vitro studies performed in brain tissues from untreated adult male rats showed that AEA increased nitric oxide synthase activity in the hypothalamus as well as in the neurohypophysis, and we showed that AEA acting through hypothalamic CB1 receptors increased OXT release [57].

Up to this time, most studies in this field focused on the role of endocannabinoids on hypothalamic oxytocinergic neurons. Nevertheless, there had been no studies aimed at understanding the role of endocannabinoid action and function in the regulation of hormones released in the neurohypophysis. It is known that TRPV1 is abundantly located in terminals of several peptidergic neurons including vasopressinergic neurons [97], and they behave as nonselective channels for several cations. AEA binds to TRPV1, generating ion currents that control neuropeptide and hormone release. Moreover, the posterior pituitary gland consists of nerve endings of hypothalamic neurons and pituicytes. Pituicytes are the major cell type in neurohypophysis and are the resident glial cells that surround these neuron terminals. Pituicytes are, therefore, critical regulators of the OXT and VP neurohypophysial output. We speculate that CB2 receptors could be located on pituicytes since they are astrocyte-like cells. In this regard, we designed experiments in order to demonstrate ECS function on the neurohypophysial level. We found an inhibitory effect of AEA on OXT and VP secretion from the neural lobe of the pituitary that seems to be mediated by NO, since the scavenging of NO by hemoglobin or the inhibition of nitric oxide synthase by L-NAME completely blocked AEA inhibition of hormone secretion. Since the putative binding site for the endocannabinoid ligand to TRPV1 is located intracellularly, then AEA must be taken up in order to reach that site. Interestingly, it has been shown that NO can stimulate AEA uptake [98]. These observations reinforce our findings that NO is necessary to mediate AEA inhibitory action on OXT and VP release from the neurohypophysis. We also demonstrated that CB2 and TRPV1 antagonists, AM630 and capsazepine respectively, completely blocked the inhibitory effects of AEA on OXT and VP release from the neurohypophysis. CB2 blockade probably affects pituicyte morphology around the nerve terminal leading to changes in neurotransmitter release and the blockade of TRPV1 confirms the participation of this channel on neurotransmitters release. Furthermore, in the presence of a CB1 receptor antagonist (AM251), the inhibitory effect of AEA persisted, suggesting that this subtype of cannabinoid receptor does not participate in OXT and VP release. In fact, some studies described that CB1 receptors are present in the

anterior pituitary gland and much less in the intermediate lobe, whereas they were not found in the neural lobe [99]. Since CB1 stimulation and TRPV1 stimulation cause opposing effects on intracellular calcium concentrations [100], it is not unreasonable to think that both receptors could not coexist functionally in the same neuron terminal. Our study shows for the first time that the ECS controls OXT and VP secretion to the periphery at the neurohypophysial level [101].

Infection has been shown to increase plasma levels of OXT and VP. A very recent article describes that female rats infected with *Toxoplasma gondii* exhibit greater abundance of mRNA for OXT and OXTR in the paraventricular nucleus of the hypothalamus as well as OXT plasma levels [102]. Also, elevated VP levels play a key role for the maintenance of osmotic, cardiovascular, and stress homeostasis during inflammatory diseases and bacterial and viral infections. Moreover, a very recent publication indicates that pronounced activation of the vasopressin system in COVID-19 patients is associated with an adverse clinical course in those patients [103]. However, the mechanism involved in the activation of both neurotransmitter-producing neurons by an immune challenge has not been deeply studied. Moreover, it was necessary to assess whether the ECS is actually involved as a hypothalamic modulator of the response of these hormones to infection. We performed in vitro studies with hypothalamic fragments from naive male rats and observed that incubation with TNF-α increased OXT production. This effect was mediated by endocannabinoids, since we observed stimulation of hypothalamic anandamide synthase activity. Moreover the presence of CB1 antagonist (AM251) completely blocked the increased hormone levels. It is known that LPS activates

magnocellular OXT and VP neurons in SON and PVN; therefore, we performed in vivo experiments where adult male rats received a single injection of LPS. We observed that LPS increased both OXT and TNF-α plasma levels after 1 hour of administration, returning to basal levels at 3 hours post-injection. Blockade of hypothalamic CB1 and CB2 by central administration of selective antagonists, AM251 and AM630, respectively, attenuated the LPS-induced increases in OXT and TNF-α plasma levels. Moreover, enhancing hypothalamic endocannabinoid signaling by brain administration of a FAAH inhibitor (URB597) potentiated LPS-induced increases in circulating OXT and TNF-α levels. The amplification of endocannabinoid signaling at the hypothalamic level thus seems to facilitate the response of neuropeptides and cytokines after an acute immune challenge [57, 89, 90, 104]. These results suggest that endocannabinoids may signal through hypothalamic cannabinoid receptors to facilitate LPS-induced neuroendocrine response during infection. (Fig. 2)

Immune Challenge and Glial Cells

As the resident macrophagic cells and main form of innate immune defense in the CNS, microglia were expected to play a crucial role after an immune challenge. Microglia are responsible for the release of chemokines and cytokines as a response to physical insults or infectious agents. Moreover, active involvement of the ECS in mediating cellular communication was observed in the functional coupling of glia and neuron synapses during normal synaptic activity and during injury. All components of the ECS are expressed in glial cells [105, 106]. During neuroinflammation, this system is highly activated,
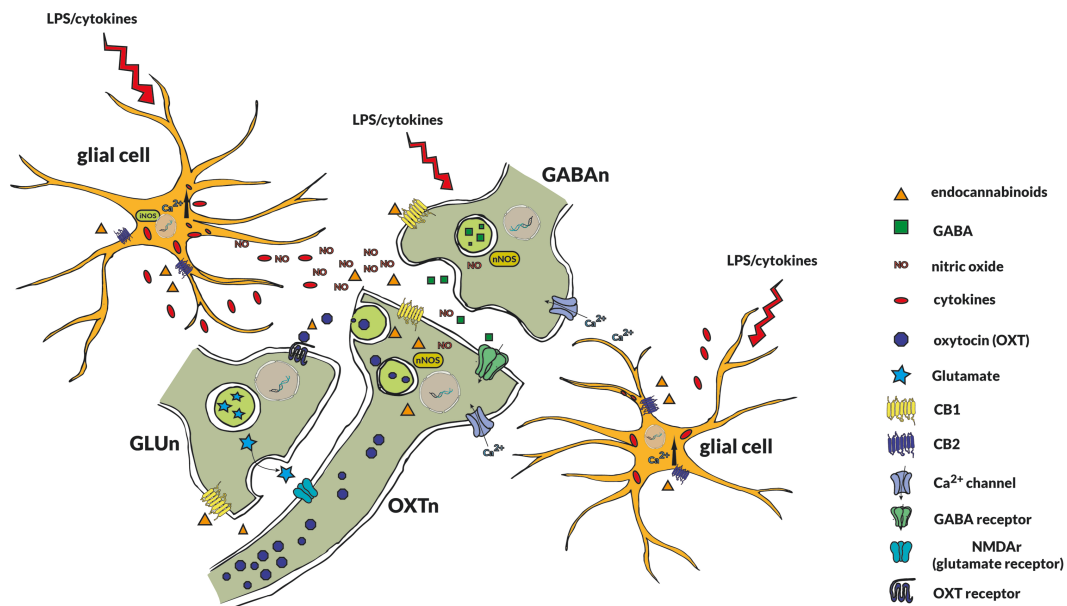

Figure 2. Putative mechanism for ECS on the release of oxytocin (OXT) from magnocellular neurons after an immune challenge. Lipopolysaccharide (LPS) and/or cytokines induce glial activation with increased Ca²⁺ production and inducible nitric oxide synthase (iNOS) activation that leads to a huge nitric oxide (NO) production. Cytokines, NO, and calcium induce the activation of magnocellular oxytocinergic neurons (OXTn) increasing the release of oxytocin (OXT) from dendrites to different adjacent neurons and from axon to general circulation via the neurohypophysis. Furthermore, inflammatory signals increase the synthesis of endocannabinoids, which stimulate the activity of the neuronal nitric oxide synthase (nNOS), increasing NO which further stimulates OXT release. Moreover, endocannabinoid signaling acts as retrograde messengers on cannabinoid receptors type 1 (CB1) present on presynaptic terminals of adjacent GABAergic (GABAn) and glutamatergic (GLUn) neurons. Since CB1 is a Gi/o coupled receptor, endocannabinoid inhibition of GABA adds to the heightened OXT release. In addition, endocannabinoid signaling also diminishes LPS-induced glutamate release, therefore preventing excitotoxic neuronal damage. Finally, endocannabinoids also bind to cannabinoid receptor type 2 (CB2) located on glial cells, downregulating glial cell activation and contributing to neuroinflammation resolution.

showing anti-inflammatory and immunomodulatory effects on the brain's innate immune response [107, 108]. Glial cells are a crucial source of de novo–produced endocannabinoids under basal conditions and during neuroinflammation.

Our group has published several original works on the activation of the ECS and the ability of these compounds to modulate the production of cytokines by microglial cells [109]. In particular, we have explored the immunomodulatory effects of AEA on the microglial response to inflammatory stimuli such as LPS and Theiler's virus [109-111]. We showed that, depending on the state of microglial activation, the effects of AEA were dependent on the activation of the CB2 receptor [110, 111] or independent of cannabinoid receptors [112]. In addition, AEA induces the production of IL-10 [113] and the increase in this anti-inflammatory cytokine has an autocrine/paracrine effect, explaining the long-term effects of AEA [114]. AEA also modulates the expression of major histocompatibility complex type II, iNOS, IL-12, IL-23, IL-1β, and TNF-α in macrophage/microglial cells infected with Theiler's virus or exposed to LPS [110, 115, 116]. Administration of AEA, a FAAH inhibitor, or an AEA reuptake inhibitor decreases spinal cord inflammation in Theiler's virus–infected mice [114]. Another study shows the molecular mechanisms involved in neuroinflammation and neurodegeneration caused by alcohol intake. In this study, we determined that ethanol modifies the expression of ECS components by epigenetic mechanisms, which would be partly responsible for the microglial activation produced by alcohol [117].

Conclusions

The interconnection between the endocrine, nervous, and immune systems is mediated by complex networks of cells and biological factors that are in constant communication to develop coordinated responses to environmental changes. This exchange of signals implies that the immune system alerts the neuroendocrine central manager when a response to infection or tissue injury is occurring. The nervous system answers with the orchestration of the neuroendocrine response with all succeeding consequences on several physiological and behavioral functions, such as sleep, mating, locomotion, and feeding. In this regard, the endocannabinoid network is suitable for multiple points of interaction with the aforementioned neuroimmunoendocrine system. Endocannabinoid levels change in response to diverse stimuli, in different developmental stages, and in a wide range of pathological conditions. ECS activation usually acts to restore physiological homeostasis by reducing inflammation, excitotoxicity, and neuronal death and controlling neuropeptide, neurotransmitter, and hormone release, among others. The ECS acts as a mediator for the communication between neurons and glial cells in order to generate the most appropriate neuroendocrine responses in each situation. Those facts indicate the physiopathological relevance of this system and why its therapeutic potential in pain, inflammation, and autoimmune and neurodegenerative disorders is receiving a great deal of attention in current research.

Acknowledgments

Funding

Disclosures

The authors have nothing to disclose in relation to the contents of this work. The authors declare no conflict of interest.

Data Availability

This is a narrative mini-review based on published data. Accordingly, specific data sharing is not applicable to this article because no datasets were generated or analyzed during the current study.

References

1. Mechoulam R, Parker LA. The endocannabinoid system and the brain. *Annu Rev Psychol*. 2013;64(1):21-47.
2. Zou S, Kumar U. Cannabinoid receptors and the endocannabinoid system: signaling and function in the central nervous system. *Int J Mol Sci* . 2018;19(3):833. Doi: 10.3390/ijms19030833
3. Hillard CJ. *The Endocannabinoid Signaling System in the CNS: A Primer*. Vol 125. 1st ed. Elsevier Inc.; 2015.
4. Pacher P, Kogan NM, Mechoulam R. Beyond THC and endocannabinoids. *Annu Rev Pharmacol Toxicol*. 2020;60:637-659.
5. Pertwee RG, ed. *Handbook of Experimental Pharmacology*. Vol 231. Springer; 2015.
6. Lu HC, Mackie K. Review of the endocannabinoid system. *Biol Psychiatry Cogn Neurosci Neuroimaging* 2021;6(6):607-615.
7. Ross R. Anandamide and vanilloid TRPV1 receptors. *Br J Pharmacol*. 2003;140(5):790-801.
8. Lu HC, MacKie K. An introduction to the endogenous cannabinoid system. *Biol Psychiatry*. 2016;79(7):516-525.
9. Di Marzo V, Wang JW. *The Endocannabinoidome: The World of Endocannabinoids and Related Mediators*. Elsevier; 2015.
10. Mackie K. Signaling via CNS cannabinoid receptors. *Mol Cell Endocrinol*. 2008;286(1-2 Suppl 1):60-65.
11. Kendall DA, Yudowski GA. Cannabinoid receptors in the central nervous system: their signaling and roles in disease. *Front Cell Neurosci*. 2017;10:294.
12. Muller C, Morales P, Reggio PH. Cannabinoid ligands targeting TRP channels. *Front Mol Neurosci*. 2019;11(January):1-15.
13. Svíženská I, Dubový P, Šulcová A. Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures - A short review. *Pharmacol Biochem Behav*. 2008;90(4):501-511.
14. Kasatkina LA, Rittchen S, Sturm EM. Neuroprotective and immunomodulatory action of the endocannabinoid system under neuroinflammation. *Int J Mol Sci*. 2021;22(11):5431. Doi: 10.3390/ijms22115431.
15. Gallego-Landin I, García-Baos A, Castro-Zavala A, Valverde O. Reviewing the role of the endocannabinoid system in the pathophysiology of depression. *Front Pharmacol*. 2021;12(Dec):1-21.

16. Hansen MK, Nguyen KT, Goehler LE, *et al*. Effects of vagotomy on lipopolysaccharide-induced brain interleukin-1β protein in rats. *Auton Neurosci Basic Clin*. 2000;85(1-3):119-126.

17. Sochocka M, Diniz BS, Leszek J. Inflammatory response in the CNS: friend or foe? *Mol Neurobiol*. 2017;54(10):8071-8089.

18. Besedovsky HO, Del Rey A. Central and peripheral cytokines mediate immune-brain connectivity. *Neurochem Res*. 2011;36(1):1-6.

19. Schwartz M, Kipnis J, Rivest S, Prat A. How do immune cells support and shape the brain in health, disease, and aging? *J Neurosci*. 2013;33(45):17587-17596.

20. Guerrero-Vargas NN, Salgado-Delgado R, Basualdo M del C, *et al*. Reciprocal interaction between the suprachiasmatic nucleus and the immune system tunes down the inflammatory response to lipopolysaccharide. *J Neuroimmunol*. 2014;273(1-2):22-30.

21. Eliava M. A new population of parvocellular oxytocin neurons controlling magnocellular neuron activity and inflammatory pain processing. *Neuron* 2016;89(6):1291-1304.

22. Bellavance MA, Rivest S. The HPA - immune axis and the immunomodulatory actions of glucocorticoids in the brain. *Front Immunol*. 2014;5(MAR):1-13.

23. Silverman MN, Sternberg EM. Glucocorticoid regulation of inflammation and its behavioral and metabolic correlates: from HPA axis to glucocorticoid receptor dysfunction. *Ann N Y Acad Sci*. 2012;1261:55-63.

24. Steiner MA, Wotjak CT. Role of the endocannabinoid system in regulation of the hypothalamic-pituitary-adrenocortical axis. *Prog Brain Res*. 2008;170(08):397-432.

25. Evanson NK, Tasker JG, Hill MN, Hillard CJ, Herman JP. Fast feedback inhibition of the HPA axis by glucocorticoids is mediated by endocannabinoid signaling. *Endocrinology* 2010;151(10):4811-4819.

26. Hill MN, McEwen BS. Involvement of the endocannabinoid system in the neurobehavioural effects of stress and glucocorticoids. *Prog Neuro-Psychopharmacol Biol Psychiatry* 2010;34(5):791-797.

27. Hill MN, Tasker JG. Review endocannabinoid signaling, glucocorticoid mediated negative feedback, and regulation of the hypothalamic-pituitary-adrenal axis. *J Neurosci*. 2012;204:5-16.

28. Akirav I. Cannabinoids and glucocorticoids modulate emotional memory after stress. *Neurosci Biobehav Rev*. 2013;37(10):2554-2563.

29. Klein TW, Newton C, Larsen K, *et al*. The cannabinoid system and immune modulation. *J Leukoc Biol*. 2003;74(4):486-496.

30. Wolfson ML, Correa F, Leishman E, *et al*. Lipopolysaccharide-induced murine embryonic resorption involves changes in endocannabinoid profiling and alters progesterone secretion and inflammatory response by a CB1-mediated fashion. *Mol Cell Endocrinol*. 2015;411:214-222.

31. Hillard CJ, Beatka M, Sarvaideo J. Endocannabinoid signaling and the hypothalamic-pituitary- adrenal axis. *Compr Physiol*; 2018;7(1):1-15.

32. Gluckman PD, Cutfield W, Hofman P, Hanson MA. The fetal, neonatal, and infant environments-the long-term consequences for disease risk. *Early Hum Dev*. 2005;81(1):51-59.

33. Kriegsfeld J, Jennings K, Bentley GE, Tsutsui K. Gonadotropin-inhibitory hormone (GnIH) and its mammalian ortholog RFamide-related peptide-3 (RFRP-3): discovery and functional implications for reproduction and stress. *J Neuroendocr* 2018;30(7):e12597.

34. Gorzalka BB, Hill MN, Chang SCH. Male-female differences in the effects of cannabinoids on sexual behavior and gonadal hormone function. *Horm Behav*. 2010;58(1):91-99.

35. Mani SK, Mitchell A, O'Malley BW. Progesterone receptor and dopamine receptors are required in Δ9-tetrahydrocannabinol modulation of sexual receptivity in female rats. *Proc Natl Acad Sci USA*. 2001;98(3):1249-1254.

36. Fernández-Solari J, Scorticati C, Mohn C, *et al*. Alcohol inhibits luteinizing hormone-releasing hormone release by activating the endocannabinoid system. *Proc Natl Acad Sci USA*. 2004;101(9):3264-3268.

37. Scorticati C, Fernández-Solari J, De Laurentiis A, *et al*. The inhibitory effect of anandamide on luteinizing hormone-releasing hormone secretion is reversed by estrogen. *Proc Natl Acad Sci USA*. 2004;101(32):11891-11896.

38. Rettori V, De Laurentiis A, Fernandez-Solari J. Alcohol and endocannabinoids: neuroendocrine interactions in the reproductive axis. *Exp Neurol*. 2010;224(1):15-22.

39. Sominsky L, Meehan CL, Walker AK, Bobrovskaya L, McLaughlin EA, Hodgson DM. Neonatal immune challenge alters reproductive development in the female rat. *Horm Behav*. 2012;62(3):345-355.

40. Ignatiuk VM, Izvolskaya MS, Sharova VS, Voronova SN, Zakharova LA. Disruptions in the reproductive system of female rats after prenatal lipopolysaccharide-induced immunological stress: role of sex steroids. *Stress* 2019;22(1):133-141.

41. McCann SM, Kimura M, Karanth S, Yu WH, Mastronardi CA, Rettori V. The mechanism of action of cytokines to control the release of hypothalamic and pituitary hormones in infection. *Ann N Y Acad Sci*. 2006;917(1):4-18.

42. De Laurentiis A, Pisera D, Lasaga M, *et al*. Effect of interleukin-6 and tumor necrosis factor-α on GABA release from mediobasal hypothalamus and posterior pituitary. *Neuroimmunomodulation*. 2000;7(2):77-83.

43. Watanabe M, Fukuda A, Nabekura J. The role of GABA in the regulation of GnRH neurons. *Front Neurosci*. 2014;8(Nov):1-9.

44. Foo YZ, Nakagawa S, Rhodes G, Simmons LW. The effects of sex hormones on immune function: a meta-analysis. *Biol Rev*. 2017;92(1):551-571.

45. Fernandez-Solari J, Prestifilippo JP, Bornstein SR, McCann SM, Rettori V. Participation of the endocannabinoid system in the effect of TNF-alpha on hypothalamic release of gonadotropin-releasing hormone. *Ann N Y Acad Sci*. 2006;1088:238-250.

46. Surkin PN, Di Rosso ME, Correa F, *et al*. Participation of hypothalamic CB1 receptors in reproductive axis disruption during immune challenge. *J Neuroendocrinol*. 2017;29(8):e12499. Doi: 10.1111/jne.12499

47. Surkin PN, Brenhouse H, Deak T, *et al*. Stress, alcohol and infection during early development: A brief review of common outcomes and mechanisms. *J Neuroendocrinol*. 2018;30(10):e12602.

48. Rettori V, Fernandez-Solari J, Prestifilippo JP, *et al*. Endocannabinoids in TNF-α and ethanol actions. *Neuroimmunomodulation*. 2007;14(3-4):188-192.

49. Watanobe H, Hayakawa Y. Hypothalamic interleukin-1β and tumor necrosis factor-α, but not interleukin-6, mediate the endotoxin-induced suppression of the reproductive axis in rats. *Endocrinology* 2003;144(11):4868-4875.

50. Sarchielli E, Comeglio P, Squecco R, *et al*. Tumor necrosis factor-α impairs kisspeptin signaling in human gonadotropin-releasing hormone primary neurons. *J Clin Endocrinol Metab*. 2017;102(1):46-56.

51. Iwasa T, Matsuzaki T, Tungalagsuvd A, *et al*. Hypothalamic Kiss1 and RFRP gene expressions are changed by a high dose of lipopolysaccharide in female rats. *Horm Behav*. 2014;66(2):309-316.

52. Claypool LE, Kasuya E, Saitoh Y, Marzban F, Ei T. N-methyl D,L-aspartate induces the release of luteinizing hormone-releasing hormone in the prepubertal and pubertal female rhesus monkey as measured by in vivo push-pull perfusion in the stalk-median eminence. *Endocrinology* 2000;141(1):219-228.

53. Bálint F, Liposits Z, Farkas I. Estrogen receptor beta and 2-arachidonoylglycerol mediate the suppressive effects of estradiol on frequency of postsynaptic currents in gonadotropin-releasing hormone neurons of metestrous mice: an acute slice electrophysiological study. *Front Cell Neurosci*. 2016;10(Mar):1-14.

54. Wilheim T, Nagy K, Mohanraj M, *et al*. Expression of type one cannabinoid receptor in different subpopulation of kisspeptin neurons and kisspeptin afferents to GnRH neurons in female mice. *Brain Struct Funct*. 2021;226(7):2387-2399.

55. Farkas I, Kalló I, Deli L, *et al*. Retrograde endocannabinoid signaling reduces GABAergic synaptic transmission to gonadotropin-releasing hormone neurons. *Endocrinology* 2010;151(12):5818-5829.

56. Farkas I, Vastagh C, Sárvári M, Liposits Z. Ghrelin decreases firing activity of gonadotropin-releasing hormone (GnRH) neurons in an estrous cycle and endocannabinoid signaling dependent manner. *PLoS One*. 2013;8(10):e781781-e781716.

57. De Laurentiis A, Fernandez-Solari J, Mohn C, Burdet B, Zorrilla Zubilete MA, Rettori V. The hypothalamic endocannabinoid system participates in the secretion of oxytocin and tumor necrosis factor-alpha induced by lipopolysaccharide. *J Neuroimmunol*. 2010;221(1-2):32-41.

58. Surkin PN, Dmytrenko G, Di Giorgio NP, Bizzozzero M, De Laurentiis A, Fernández-Solari J. Participation of TRPV1 in the activity of the GnRH system in male rats. *Eur J Neurosci*. 2020;52(3):2995-3001.

59. Devesa I, Planells-Cases R, Fernández-Ballester G, González-Ros JM, Ferrer-Montiel A, Fernández-Carvajal A. Role of the transient receptor potential vanilloid 1 in inflammation and sepsis. *J Inflamm Res* 2011;4(1):67-81.

60. Yoshida A, Furube E, Mannari T, *et al*. TRPV1 is crucial for proinflammatory STAT3 signaling and thermoregulation-associated pathways in the brain during inflammation. *Sci Rep*. 2016;6(April):1-11.

61. Yang Y, Yang H, Wang Z, *et al*. Cannabinoid receptor 1 suppresses transient receptor potential vanilloid 1-induced inflammatory responses to corneal injury. *Cell Signal*. 2013;25(2):501-511.

62. Turnbull AV, Rivier CL. Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiol Rev*. 1999;79(1):1-71.

63. Khazaeipool Z, Wiederman M, Inoue W. Prostaglandin E2 depresses GABA release onto parvocellular neuroendocrine neurones in the paraventricular nucleus of the hypothalamus via presynaptic receptors. *J Neuroendocrinol*. 2018;30(11):e126381-e126310.

64. Sharma A, Steven S, Bosmann M. The pituitary gland prevents shock-associated death by controlling multiple inflammatory mediators. *Biochem Biophys Res Commun*. 2019;509(1):188-193.

65. Clodi M, Vila G, Geyeregger R, *et al*. Oxytocin alleviates the neuroendocrine and cytokine response to bacterial endotoxin in healthy men. *Am J Physiol Endocrinol Metab*. 2008;295(3):E686-E691. Doi: 10.1152/ajpendo.90263.2008

66. Vargas-Martínez F, Uvnäs-Moberg K, Petersson M, Olausson HA, Jiménez-Estrada I. Neuropeptides as neuroprotective agents: oxytocin a forefront developmental player in the mammalian brain. *Prog Neurobiol*. 2014;123:37-78.

67. Wang P, Yang HP, Tian S, *et al*. Oxytocin-secreting system: a major part of the neuroendocrine center regulating immunologic activity. *J Neuroimmunol*. 2015;289:152-161.

68. Amini-Khoei H, Mohammadi-Asl A, Amiri S, *et al*. Approaches mediating oxytocin regulation of the immune system. *Front Immunol*. 2017;7(Jan):1-9.

69. Kingsbury MA, Bilbo SD. The inflammatory event of birth: how oxytocin signaling may guide the development of the brain and gastrointestinal system. *Front Neuroendocrinol*. 2019;55:100794. Doi: 10.1016/j.yfrne.2019.100794

70. D'Mello C, Swain MG. Immune-to-brain communication pathways in inflammation-associated sickness and depression. *Curr Top Behav Neurosci* 2017;31:73-94.

71. Di S, Popescu IR, Tasker JG. Glial control of endocannabinoid heterosynaptic modulation in hypothalamic magnocellular neuroendocrine cells. *J Neurosci*. 2013;33(46):18331-18342.

72. Kelley KW, McCuskera RH. Getting nervous about immunity. *Semin Immunol*. 2014;26(5):389-393.

73. Chini B, Fanelli F. Molecular basis of ligand binding and receptor activation in the oxytocin and vasopressin receptor family. *Exp Physiol*. 2000;85(Special Issue):59S-66S. Doi: 10.1111/j.1469-445x.2000.tb00008.x

74. Jurek B, Neumann ID. The oxytocin receptor: from intracellular signaling to behavior. *Physiol Rev*. 2018;98(3):1805-1908.

75. Zhimin S. Cross-talk among oxytocin and arginine-vasopressin receptors: relevance for basic and clinical studies of the brain and periphery. *Front Neuroendocr* 2018;51:14-24.

76. Quintana DS, Guastella AJ. An allostatic theory of oxytocin. *Trends Cogn Sci*. 2020;24(7):515-528.

77. Guzzi F, Zanchetta D, Cassoni P, *et al*. Localization of the human oxytocin receptor in caveolin-1 enriched domains turns the receptor-mediated inhibition of cell growth into a proliferative response. *Oncogene* 2002;21(11):1658-1667.

78. Accialini P, Etcheverry T, Malbrán MN, Leguizamón G, Maté S, Farina M. Anandamide regulates oxytocin/oxytocin receptor system in human placenta at term. *Placenta* 2020;93(Nov):23-25.

79. Accialini P, Etcheverry T, Abán C, Negri Malbrán M, Leguizamón G, Maté SF, Farina M. The endocannabinoid anandamide regulates oxytocin receptor in human placenta. *Placenta J* 2019;83:e1-118.

80. Hagberg H, Mallard C, Ferriero DM, *et al*. The role of inflammation in perinatal brain injury. *Nat Rev Neurol*. 2015;11(4):192-208.

81. Andari E, Nishitani S, Kaundinya G, *et al*. Epigenetic modification of the oxytocin receptor gene: implications for autism symptom severity and brain functional connectivity. *Neuropsychopharmacology*. 2020;45(7):1150-1158.

82. Kenkel WM, Perkeybile AM, Yee JR, *et al*. Behavioral and epigenetic consequences of oxytocin treatment at birth. *Sci Adv*. 2019;5(5):1-10.

83. Perkeybile AM, Carter CS, Wroblewski KL, *et al*. Early nurture epigenetically tunes the oxytocin receptor. *Psychoneuroendocrinology* 2019;99:128-136.

84. Mairesse J, Zinni M, Pansiot J, *et al*. Oxytocin receptor agonist reduces perinatal brain damage by targeting microglia. *Glia* 2018(June):1-15.

85. McKay EC, Beck JS, Khoo SK, *et al*. Peri-infarct upregulation of the oxytocin receptor in vascular dementia. *J Neuropathol Exp Neurol*. 2019;78(5):436-452.

86. Gulliver D, Werry E, Reekie TA, Katte TA, Jorgensen W, Kassiou M. Targeting the oxytocin system: new pharmacotherapeutic approaches. *Trends Pharmacol Sci*. 2019;40(1):22-37.

87. Uvnäs Moberg K, Handlin L, Kendall-Tackett K, Petersson M. Oxytocin is a principal hormone that exerts part of its effects by active fragments. *Med Hypotheses*. 2019;133(September):109394.

88. Sue Carter C, Kenkel WM, Maclean EL, *et al*. Is oxytocin "nature's medicine"? *Pharmacol Rev*. 2020;72(4):829-861.

89. De Laurentiis A, Fernández Solari J, Mohn C, *et al*. Endocannabinoid system participates in neuroendocrine control of homeostasis. *Neuroimmunomodulation*. 2010;17(3):153-156.

90. De Laurentiis A, Araujo HA, Rettori V. Role of the endocannabinoid system in the neuroendocrine responses to inflammation. *Curr Pharm Des*. 2014;20(29):4697-4706.

91. Crowe MS, Nass SR, Gabella KM, Kinsey SG. The endocannabinoid system modulates stress, emotionality, and inflammation. *Brain Behav Immun*. 2014;42:1-5.

92. Tasker JG, Chen C, Fisher MO, Fu X, Rainville JR, Weiss GL. *Endocannabinoid Regulation of Neuroendocrine Systems*. Vol 125. 1st ed. Elsevier Inc.; 2015.

93. Herkenham M, Lynn B, Johnson MR, Melvin LS, de Costa BR, Rice KC. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J Neurosci*. 1991;11(2):563-583.

94. Scorticati C, Mohn C, De Laurentiis A, *et al*. The effect of anandamide on prolactin secretion is modulated by estrogen. *Proc Natl Acad Sci USA*. 2003;100(4):2134-2139.

95. Sabatier N, Caquineau C, Dayanithi G, *et al*. Alpha-melanocyte-stimulating hormone stimulates oxytocin release from the dendrites of hypothalamic neurons while inhibiting oxytocin release from their terminals in the neurohypophysis. *J Neurosci*. 2003;23(32):10351-10358.

96. McDonald NA, Kuzmiski JB, Naderi N, Schwab Y, Pittman QJ. Endogenous modulators of synaptic transmission: cannabinoid regulation in the supraoptic nucleus. *Prog Brain Res*. 2008;170(08):129-136.

97. Sudbury JR, Ciura S, Sharif-Naeini R, Bourque CW. Osmotic and thermal control of magnocellular neurosecretory neurons – role of an N-terminal variant of trpv1. *Eur J Neurosci.* 2010;32(12):2022-2030.

98. Maccarrone M, Bari M, Lorenzon T, Bisogno T, Di Marzo V, Finazzi-Agrò A. Anandamide uptake by human endothelial cells and its regulation by nitric oxide. *J Biol Chem.* 2000;275(18):13484-13492.

99. Wenger T, Fernández-Ruiz JJ, Ramos JA. Immunocytochemical demonstration of CB1 cannabinoid receptors in the anterior lobe of the pituitary gland. *J Neuroendocrinol.* 1999;11(11):873-878.

100. Szallasi A, Di Marzo V. New perspectives on enigmatic vanilloid receptors. *Trends Neurosci.* 2000;23(10):491-497.

101. Luce V, Fernandez Solari J, Rettori V, De Laurentiis A. The inhibitory effect of anandamide on oxytocin and vasopressin secretion from neurohypophysis is mediated by nitric oxide. *Regul Pept.* 2013;188:31-39.

102. Abdulai-Saiku S, Vyas A. Toxoplasma gondii infection causes an atypical abundance of oxytocin and its receptor in the female rat brain. *Pathogens* 2021;10(11):14951-14957.

103. Gregoriano C, Molitor A, Haag E, *et al*. Activation of vasopressin system during COVID-19 is associated with adverse clinical outcomes: an observational study. *J Endocr Soc.* 2021;5(6):1-10.

104. Surkin PN, Gallino SL, Luce V, Correa F, Fernandez-Solari J, De Laurentiis A. Pharmacological augmentation of endocannabinoid signaling reduces the neuroendocrine response to stress. *Psychoneuroendocrinology* 2018;87:131-140.

105. Stella N. Endocannabinoid signaling in microglial cells. *Neuropharmacology* 2009;56(Suppl 1):244-253.

106. Stella N. Cannabinoid and cannabinoid-like receptors in microglia, astrocytes and astrocytomas. *Bone* 2011;23(1):1-7.

107. Navarrete M, Díez A, Araque A. Astrocytes in endocannabinoid signalling. *Philos Trans R Soc Lond B Biol Sci* 2014;369(1654):20130599.

108. Lisboa SF, Gomes FV, Guimaraes FS, Campos AC. Microglial cells as a link between cannabinoids and the immune hypothesis of psychiatric disorders. *Front Neurol.* 2016;7(JAN):5.

109. Correa FG, Mestre L, Docagne F, Borrell J, Guaza C. Chapter 9 The endocannabinoid anandamide. from immunomodulation to neuroprotection. implications for multiple sclerosis. *Vitam Horm.* 2009;81(C):207-230.

110. Correa F, Mestre L, Docagne F, Guaza C. Activation of cannabinoid CB 2 receptor negatively regulates IL-12p40 production in murine macrophages: role of IL-10 and ERK1/2 kinase signaling. *Br J Pharmacol.* 2005;145(4):441-448.

111. Correa F, Docagne F, Mestre L, *et al*. A role for CB2 receptors in anandamide signalling pathways involved in the regulation of IL-12 and IL-23 in microglial cells. *Biochem Pharmacol.* 2009;77(1):86-100.

112. Correa F, Docagne F, Clemente D, Mestre L, Becker C, Guaza C. Anandamide inhibits IL-12p40 production by acting on the promoter repressor element GA-12: possible involvement of the COX-2 metabolite prostamide E 2. *Biochem J.* 2008;409(3):761-770.

113. Correa F, Hernangómez M, Mestre L, *et al*. Anandamide enhances IL-10 production in activated microglia by targeting CB 2 receptors: roles of ERK1/2, JNK, and NF-κB. *Glia* 2010;58(2):135-147.

114. Correa F, Hernangómez-Herrero M, Mestre L, Loría F, Docagne F, Guaza C. The endocannabinoid anandamide downregulates IL-23 and IL-12 subunits in a viral model of multiple sclerosis: evidence for a cross-talk between IL-12p70/IL-23 axis and IL-10 in microglial cells. *Brain Behav Immun.* 2011;25(4):736-749.

115. Mestre L, Correa F, Arévalo-Martín A, *et al*. Pharmacological modulation of the endocannabinoid system in a viral model of multiple sclerosis. *J Neurochem.* 2005;92(6):1327-1339.

116. Mestre L, Iñigo PM, Mecha M, *et al*. Anandamide inhibits Theiler's virus induced VCAM-1 in brain endothelial cells and reduces leukocyte transmigration in a model of blood brain barrier by activation of CB1 receptors. *J Neuroinflammation.* 2011;8(1):102.

117. Correa F, De Laurentiis A, Franchi AM. Ethanol downregulates N- acyl phosphatidylethanolamine- phospholipase D expression in BV2 microglial cells via epigenetic mechanisms. *Eur J Pharmacol.* 2016;786:224-233.