Western Norway University of Applied Sciences

BACHELOR THESIS

Mechanisms of IL-1 in the Brain Related to Fever and Depression

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Abstract

Several studies have reported that the proinflammatory cytokine, interleukin-1 (IL-1), promotes sickness feeling, fever and even depression. This short review article is composed using both original- and review articles, and it describes the common molecular network that controls the onset and development of these conditions. IL-1 plays an important role in the immune response and the central nervous system (CNS) by activating a proinflammatory signaling pathway. The signaling cascade begin when IL-1 binds to its specific receptor called interleukin-1 receptor (IL-1R). This complex can further via the gene transcription regulator called nuclear factor kappa-light-chain-enhancer and activated B-cells (NFκB) activate the cascade that results in further production of IL-1, an upregulation of cyclooxygenase-2 (COX-2) and an activation of the hypothalamic-pituitary-adrenal (HPA) axis. COX-2 can further be connected to the development of fever because of its production of prostaglandin E_2 (PGE_2). The hyperactivity of the HPA axis and the effect of IL-1 in the monoaminergic neurotransmitter system can be linked to depression. Growing evidence from several studies link increased and activated immune signaling caused by IL-1, to sickness feeling, fever and it also seems to play a role in the pathophysiology of depression.

Key words: Interleukin-1, sickness feeling, fever, depression, hypothalamic-pituitary-adrenal axis

Preface

This bachelor thesis was written in collaboration with the Faculty of Science at Eötvös Loránd University, Budapest, Hungary and the Faculty of Engineering and Natural Sciences at Western Norway University of Applied Sciences, Bergen, Norway. The project was supposed to be executed as part of a 12-week exchange program at Eötvös Loránd University, but due to the coronavirus pandemic our stay was cut short and our project was converted to a literary study. However, the project has been very educational, and we are grateful for the opportunity.

We would like to thank our supervisor Gábor Juhász from the Research Group of Proteomics at Eötvös Loránd University for guiding us through this project and supplying us with relevant review- and original articles. We would also like to thank our Norwegian supervisor, Alvhild Alette Bjørkum at the Faculty of Engineering and Natural Sciences at Western Norway University of Applied Sciences for giving us this opportunity, and for supporting and helping us along the way. Lastly, we would like to thank Turid Aarhus Braaseth for preparing us for the exchange program and for guiding us on the group process.

29th of May 2020

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Introduction

Proinflammatory Cytokines

The organism is controlled by two main systems, the immune system and the nervous system, which have common and different molecular mechanisms. The human immune system is a complex system that protects the body from pathogenic microbes and proteins from unknown origin. One part of this complex system are cytokines who also have functional roles in the nervous system. Cytokines are mostly low molecular weight proteins or glycoproteins which act as a chemical means of communication between cells (Lea, 2006). They play a key role in the regulation of the immune response and are excreted from a wide range of cells in the innate immune system including lymphocytes and monocytes. In addition to immune cells, there are non-immune system cells in the central nervous system (CNS) that can release cytokines, for example microglia and astrocytes. The cells must be activated to produce cytokines. This activation can happen through other cells of the immune system or through pattern recognizing receptors (PRR) which recognize parts of unknown microbes, such as pathogen-associated molecular patterns (PAMPs). PAMPs are characteristic structural properties of bacteria, fungi, viruses and the like. When the cells have been activated, they will begin to produce cytokines with inflammatory activity.

Both function and structure are used to categorize cytokines (Lea, 2006). Some examples of cytokine families are interferons, interleukins, the tumor necrosis factor family and colony stimulating agents. Each family of cytokines in turn consists of many different cytokines that have different functions.

Cytokines do not work as effector molecules by themselves, they act only after binding to specific surface receptors in the membrane of cells (Lea, 2006). Although the cytokines bind to their specific surface receptors, they often have partially overlapping effects and affect each other's activity. It has also been shown that different cytokines can affect the production of other cytokines, and they can regulate the levels of each other's membrane receptors. It is therefore believed that cytokines form part of a functional network where each individual cytokine can influence the synthesis and action of several other cytokines.

The following article will describe the influence the proinflammatory cytokine interleukin-1 can have on the brain during a pre-inflammatory process (Dantzer, 2009). Normal physiology in the brain consists of cytokines in large quantities. Astrocytes and microglia, which both

produce cytokines, participate in neuroinflammatory disease progression (Liu, Buisman-Pijlman, & Hutchinson, 2014). In rare and severe pathological conditions, the inflammatory process might develop into a neurodegenerative disorder in the brain. However, IL-1 can through neurophysiological mechanisms be connected to sickness feeling, fever and depression.

The Proinflammatory Cytokine Interleukin-1

Growing evidence from several studies described by Roerink et al. (2017) and Farooq et al. (2017) show that IL-1 in the CNS can play an important role in the development of sickness feeling, fever and depression. The IL-1 family consists of 11 members that play a key role in the regulation of the immune response during for example tissue injury and infections (Pasic, Levy, & Sullivan, 2003; Roerink et al., 2017). This short review article is focused on the group of proinflammatory cytokines called interleukin-1α and β (IL-1α and IL-1β). IL-1 binds to its specific receptor called interleukin-1 receptor (IL-1R). This complex can further via the gene transcription regulator called nuclear factor kappa-light-chain-enhancer and activated B-cells (NFκB) activate a signaling cascade and cause a proinflammatory signal that in the end can be connected to sickness feeling, fever and depression.

Fever

Fever is one of the oldest clinical indicators of disease in mammals, and it often occurs in response to infection, inflammation and trauma (Ogoina, 2011). Already at the end of the 19th century it was demonstrated that fever required the involvement of the brain (Atkins, 1982). It was also understood at that time that the inflammatory process resulted in the release of substances that produced fever. These substances were later identified as cytokines (Dinarello, 2015). Fever is usually defined as a regulated rise in body temperature above normal daily fluctuations occurring in conjunction with an elevated thermoregulatory set point (Ogoina, 2011). Apart from this, fever is also accompanied by various sickness feelings, changes in metabolic and physiological characteristics of body systems, and alterations in immune responses. An individual with fever may display a range of behavioral changes, such as anorexia, fatigue, loss of interest in daily activities, social withdrawal, sleep disturbances and cognitive dysfunction, collectively termed “sickness feeling” (Harden, Kent, Pittman, & Roth, 2015). The febrile response is orchestrated by the central nervous system (CNS) through endocrine, neurological, immunological and behavioral mechanisms.

Peripheral and centrally generated temperature signals are received and integrated in the preoptic area (POA) of the anterior hypothalamus. This part of the brain is considered as the major thermoregulatory center in the CNS (Ogoina, 2011). Warm-sensitive neurons are located in the POA, while cold-sensitive neurons are located in the posterior hypothalamic area (PHA) (Felten, 2003). These warm- and cold-sensitive neurons are activated or inhibited in response to temperature changes. The POA initiates neuronal responses for heat dissipation, while the PHA initiates neuronal responses for heat generation. Neural pathways arising from the brain stem and the limbic forebrain areas, can modulate the activity of these thermoregulatory systems. The POA is also responsive to pyrogens and the proinflammatory cytokine IL-1. IL-1 can activate a brain network in the POA that can generate an increased thermoregulatory setpoint, and so, initiate a disease-associated fever.

Depression

Depression is a common disorder that is believed to become the leading cause of disability worldwide by the year 2030 (Mathers & Loncar, 2006). The disorder is characterized by hyperactivity of the hypothalamus-pituitary-adrenal (HPA) axis and abnormalities in the central monoaminergic neurotransmitter system resulting in behavioral changes and alterations in neurohormonal pathways (Pasic et al., 2003). An increased level of IL-1 in the CNS can affect both the activity of the HPA axis which is considered to be secondary to the hyperactivity of corticotrophin-releasing hormone (CRH) and the metabolism of serotonin and dopamine which are essential for the regulations of emotions (Raison, Capuron, & Miller, 2006).

There are no satisfactory treatments for depression on the market today (Pasic et al., 2003). Therefore, this subject needs more investigation to better understand the molecular network that regulates and causes depression. Studies show that depression is associated with alterations in immune function. During an inflammation, cytokines will be excreted peripherally and eventually reach the CNS via a humoral or a neural communication pathway. Alterations in the CNS caused by various proinflammatory cytokines, such as IL-1, can in some cases explain the onset and development of depression (Farooq et al., 2017; Roerink et al., 2017).

IL-1 can affect the body in many ways and several studies show that it can impact the CNS. This short review article will try to compose a common molecular network that controls sickness feeling, fever and depression.

Material and Methods

In this bachelor thesis both review- and original research articles were used to compose a short review article. The thesis is primarily based on review- and original research articles handed out by Dr. Gábor Juhász from the Research Group of Proteomics, Institute of Biology, Faculty of Science at Eötvös Loránd University, Budapest, Hungary and Dr. Alvhild Alette Bjørkum at the Department of Safety, Chemistry and Biomedical Laboratory Sciences at the Faculty of Engineering and Natural Sciences at Western Norway University of Applied Sciences, Bergen, Norway. Some of the review- and original articles used as sources and referred to in the articles handed out were also used and referred to here. Additional literature database searches in PubMed were used as well. The following terms were used in different combinations in the literature searches: “IL-1”, “toll-like-receptor 4”, “depression”, “fever” and “fever mechanisms”.

Results

The Proinflammatory Interleukin-1 Cascade Reaction, Including TLR4, COX-2 and NFκB

Interleukin-1 (IL-1) is part of a proinflammatory signaling pathway that leads to further production of IL-1, an upregulation of cyclooxygenase-2 (COX-2) and activation of the hypothalamic-pituitary-adrenal (HPA) axis (Dantzer, 2009; Liu et al., 2014). An increase of COX-2 and IL-1 depends on the activation of the gene transcription regulator called nuclear factor kappa-light-chain-enhancer of activated B-cells (NFκB) (Roerink et al., 2017). The activation of NFκB is triggered by an extracellular stimulus that binds to a receptor in the interleukin-1 receptor (IL-1R) family, such as toll-like receptor 4 (TLR4). A microbial product (lipopolysaccharides (LPS)) or a proinflammatory cytokine (IL-1) are examples of extracellular stimulus.

Peripherally, TLRs are expressed in the membrane of innate immune cells, such as monocytes and macrophages, while in the central nervous system (CNS) they are predominantly expressed on microglia (Dantzer, 2009; Liu et al., 2014). The receptors recognize microbial molecular patterns like pathogen-associated molecular patterns (PAMPs), including LPS which is a component of the membrane in Gram-negative bacteria (Lawrence, 2009). Binding of for example LPS to TLR4 or IL-1β to IL-1R1 makes a complex that triggers intracellular proinflammatory transcriptions via the adaptor protein Myeloid differentiation primary response 88 (MyD88) (Liu et al., 2014) (*Fig. 1*). Dimerization of MyD88 leads to a recruitment and phosphorylation of the interleukin-1 receptor-associated kinases 1 and 2 (IRAK1 and IRAK2) (Parnet, Kelley, Bluthé, & Dantzer, 2002). IRAK1 will further interact with TNF receptor-associated factor 6 (TRAF-6), resulting in an activation and translocation of NFκB to the nucleus. NFκB binds to the target-gene in the nucleus resulting in a messenger RNA (mRNA)-product coding for the proteins IL-1 and COX-2.

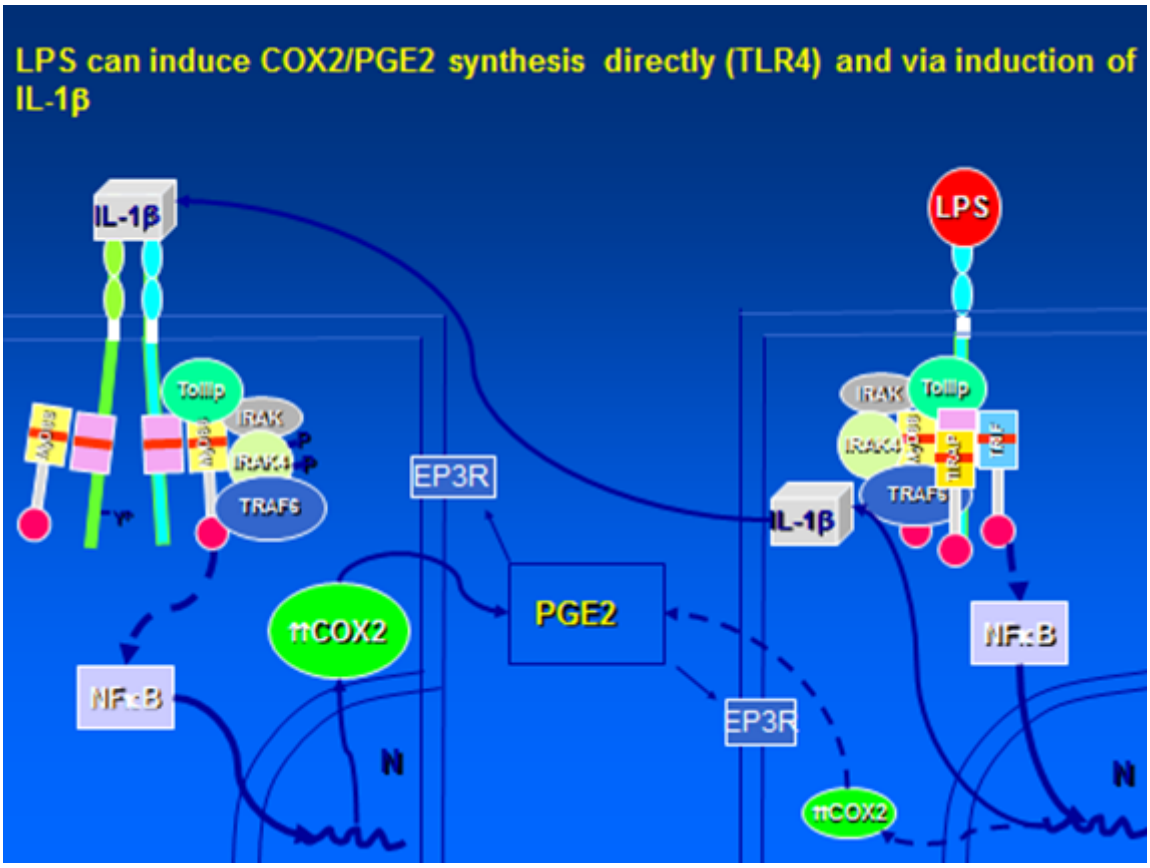

Figure 1 – The Proinflammatory Interleukin-1 Cascade Reaction (G. Juhász, personal communication, April 2nd 2020). Lipopolysaccharide (LPS) can induce cyclooxygenase-2 (COX-2) and prostaglandin E2-group (PGE2) synthesis directly by binding to toll-like receptor 4 (TLR4) and via induction of interleukin-1β (IL-1β). Binding of LPS to TLR4 or IL-1β to interleukin-1 receptor (IL-1R1) leads to an inflammatory response starting with dimerization of the adaptor protein Myeloid differentiation primary response 88 (MyD88). Dimerization of MyD88 leads to a recruitment and phosphorylation of the interleukin-1 receptor-associated kinases 1 and 2 (IRAK1 and IRAK2). IRAK1 will further interact with TNF receptor-associated factor 6 (TRAF-6), resulting in an activation and translocation of NFκB to the nucleus. This activation leads to an upregulation of IL-1 β and COX-2. COX-2 will along with prostaglandin E catalyze the synthesis of the PGE2. PGE2 will act on its specific prostaglandin EP3 receptor.

COX-2 is an enzyme that along with prostaglandin E synthase catalyze the synthesis of the prostaglandin E_2-group (PGE_2) (Dantzer, 2009). PGE_2 is a small molecule working as the main mediator of cytokine-induced fever and it can also activate the HPA axis (Dantzer, 2009; Ogoina, 2011). PGE_2 will diffuse across the blood-brain barrier (BBB) and into the brain tissue where it will further act on its specific prostaglandin EP_3 receptors located in the brain stem and hypothalamic neural structures. Binding of PGE_2 to its specific EP_3 receptor makes a complex that controls the HPA axis activity and regulates the body temperature. The connection between the activation of the HPA axis, depression and fever will be described later in this short review article.

How IL- 1 can Circumvent the Blood-Brain Barrier

Cytokines are unlikely to cross the BBB because of their "large" size (Pasic et al., 2003; Roerink et al., 2017). However, several review articles describe that IL-1 are able to reach or send signals to the central nervous system (CNS) via various mechanisms (Dantzer, 2009; Pasic et al., 2003). Peripherally produced IL-1 can affect the brain via two main communication pathways: (1) a humoral pathway and (2) a neural pathway (Dantzer, 2009; Roerink et al., 2017). Also, a combination of these two pathways has been described where the communication ways "work together" to activate the immunocompetent cells of the brain, the microglia. These cells can further produce IL-1 locally.

The humoral pathway involves the production of IL-1 by phagocytic cells in the choroid plexus which is located in the ependyma of the circumventricular organs (CVOs) (Dahl & Rinvik, 2010). The choroid plexus consists of many small blood vessels, and it is responsible for the production of the cerebrospinal fluid (CSF). IL-1 diffuses through the fenestrated endothelium to brain cells expressing IL-1Rs. This means that the BBB can be bypassed via the areas surrounding the CVOs because of its high permeability. IL-1 can also be produced locally in the brain by activated macrophages and perivascular endothelial cells. The neural pathway is represented by the vagus nerve and afferent neurons that innervate the infected part of the body. The vagus nerve consists of nerves containing sensory, somato motor and autonomous ability, and is the main connection between the CNS and the major organs (Dahl & Rinvik, 2010; Sand, Sjaastad, Haug, & Bjålie, 2006). Cytokine signals can mainly via this nerve affect the relevant brain regions directly.

IL-1 Receptor Distribution in the Brain

IL-1 Receptors

Several receptor subtypes have been described to mediate the effect of IL-1 on its cellular targets (Dantzer, 2009). The interleukin-1 receptor (IL-1R) family is composed of eight members that respond to IL-1 and IL-18 (Parnet et al., 2002). These receptors feature an extracellular domain with three immunoglobulin-like domains and an intracellular toll domain that is connected to adaptor proteins and kinase cascades.

Two of the subtypes of IL-1 receptors that have been identified are the type 1 receptor (IL-1R1) and the type 2 receptor (IL-1R2). IL-1R1 is expressed on for example T-cells and

fibroblasts and the IL-1R2 is produced mainly from basal epithelial cells of the skin, urethra and vagina (Ericsson, Liu, Hart, & Sawchenko, 1995). One of the important functions of IL-1R1 is to mediate the biological effect of IL-1 leading to an activation of the transcription factor NFκB (Dantzer, 2009). In contrast, IL-1R2, acts like a decoy receptor that negatively regulates the IL-1 system.

Localization of the IL-1 Receptor

Human studies on IL-1R1 distribution in the brain are scarce (Roerink et al., 2017). Therefore, studies on rats or mice are utilized. Katsuura et.al (1988) were the first investigators that localized IL-1 receptors, and their findings indicated a neuronal localization. Later studies have proved that IL-1 receptors are spread widely across the brain, mostly located in the granular layer of the dentate gyrus in the hippocampus, in the granule cell layer of the cerebellum, in the hypothalamus and in the pyramidal cell layer of the hippocampus (Dantzer, 2009). By using in situ hybridization, IL-1R messenger RNA (mRNA) was identified in the anterior olfactory nucleus, medial thalamic nucleus, posterior thalamic nucleus, basolateral amygdaloid nucleus, ventromedial hypothalamus, arcuate nucleus, medial eminence, mesencephalic trigeminal nucleus, motor trigeminal nucleus, facial nucleus and Purkinje cells of the cerebellum (Pasic et al., 2003). In addition, Ericsson et al. (1995) showed IL-1R1 mRNA expression in non-neuronal cells in structures at the interface between the brain parenchyma and its fluid environments, such as the choroid plexus and the endothelial cells of the brain vasculature by using the same method. Neuronal expression appeared mostly in the hippocampus, but it was also detected in a few cell groups of the basolateral nucleus of the amygdala and the basomedial nuclei of the hypothalamus.

It is well known that the density of cytokines arises in much larger quantities during an infection. However, the expression of IL-1 has also been identified in neurons and glial cells in the CNS during noninflammatory environments by histochemical studies (Pasic et al., 2003).

In contrast to the research of the IL-1R1 distribution in the rat brain, the research regarding expression of IL-1R2 mRNA is less known. The expression of IL-1R2 have been restricted to brain endothelial cells and infiltrating neutrophils (Dantzer, 2009).

When investigators characterize IL-1 receptors on neurons, glial cells and endothelial cells of brain venules with the use of biochemical techniques, they have proved that the IL-1 receptors show similarity to the receptors expressed peripherally on both non-immune and

immune cells. An explanation could be that the majority of the IL-1R family has been cloned from blood cell lineages (Dantzer, 2009). To determine the type and localization of IL-1 receptors in the brain, an autoradiographic method was used to detect radioiodinated ligands, such as IL-1α and IL-1β. Other methods used for determination are polymerase-chain reaction (PCR) that can multiply a fragment of the complementary DNA-encoding IL-1 receptor and immunohistochemical detection with antibodies raised against epitopes of IL-1 receptors (Dantzer, 2009; Parnet et al., 2002). These methods cannot conclusively determine if the receptors in the brain and those peripherally are identical. However, detection of IL-1 receptors in the brain using in situ hybridization indicates that the same IL-1R1 mRNA is present peripherally and in central nervous tissue.

The activation of IL-1R1 leads to an intracellular pathway in the brain which is similar to that in the periphery (Roerink et al., 2017). Even though IL-1β is injected into the brain or at the periphery, it results in the same behavioral effects. However, the effects of IL-1β take longer to develop and dissipate quicker with central injection. This arouses attention to the functionality of the IL-1Rs in the brain (Parnet et al., 2002). An experiment showed an increased level of IL-1β mRNA in the hypothalamus directly after peripheral injection of IL-1β (Masanori et al., 2014). The concentration in the hypothalamus decreased within 24 hours, but an upregulation of IL-1β mRNA persisted in the cerebral cortex. This was accompanied by a decrease in spontaneous activity lasting for several days (Roerink et al., 2017). An explanation of the IL-1β transcription can be due to the epigenetic changes in microglial cells that can play a role in neuroinflammatory disorders.

Hypothalamus-Pituitary-Adrenal Axis Activation

IL-1 is able to activate the hypothalamus-pituitary-adrenal (HPA) axis through two main pathways: (1) through reducing negative feedback on HPA signaling, and (2) by directly stimulating HPA activation in the hypothalamus. Cytokines also have the ability to amplify the feed-forward signaling within the HPA axis (Liu et al., 2014).

When activated, the HPA axis works to restore homeostasis following different stressors. Stress refers to a challenge to the body's homeostatic state, and can be classified broadly as psychological, physiological and immunological in origin (Liu et al., 2014). Toll-like receptor 4 (TLR4) activation, for example, is considered as an immunological stressor. Stress

will activate the HPA axis, which forms the neuroendocrine stress response. Activation of the HPA axis begins with neurons in the paraventricular nucleus (PVN) of the hypothalamus and leads to secretion of corticotropin-releasing hormone (CRH), which again stimulates the anterior pituitary gland to produce and release adrenocorticotropic hormone (ACTH) into the blood stream (*Fig. 2*). Upon binding to melanocortin 2 receptors expressed on the adrenal cortex, ACTH stimulates glucocorticoid (GC) production (Liu et al., 2014). One important GC is cortisol which is classified as a stress hormone that inhibits the effects of CRH and the production and secretion of CRH from the hypothalamus through a negative-feedback mechanism (Bishop, Fody, & Schoeff, 2018). It can also have an immunosuppressive effect.

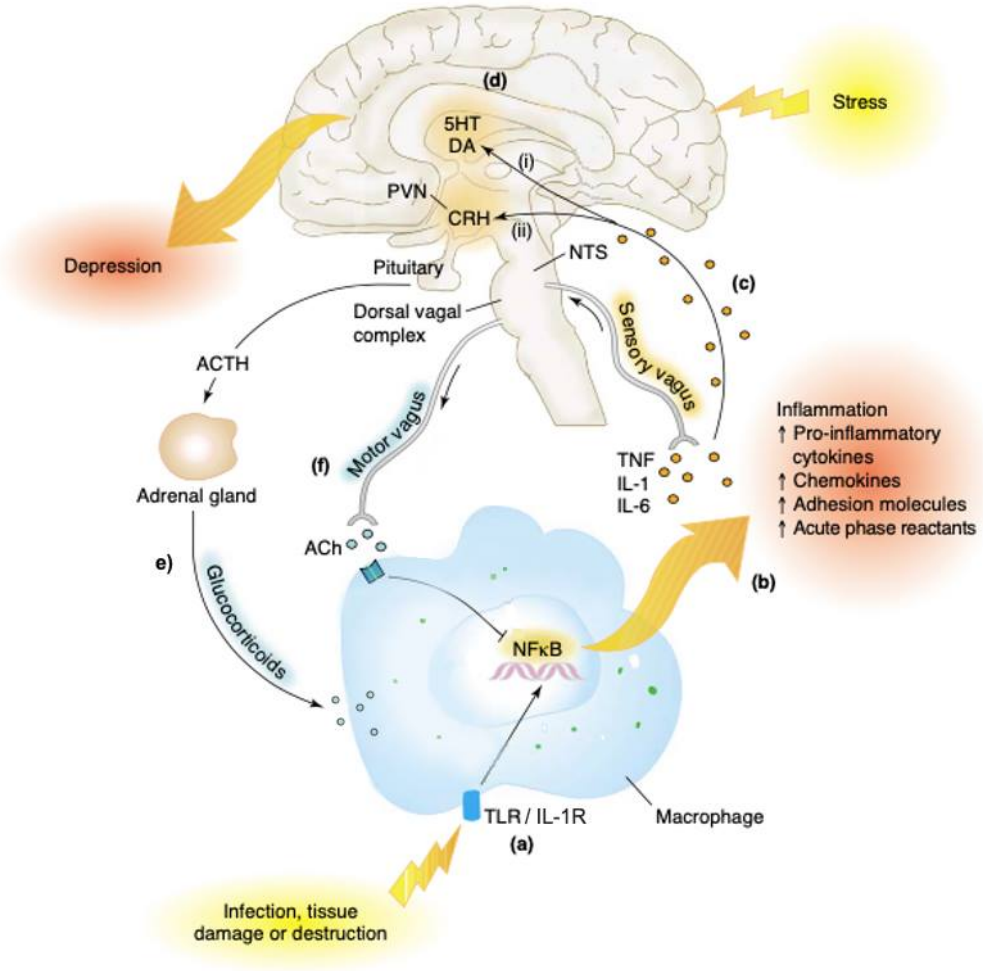

Figure 2 – Stress-Immune Interactions and Depression (Modified from Raison et al. (2006) with permission). a) The activation of NFκB is triggered by an extracellular stimulus that binds to TLR4 during inflammation. b) This leads to a release of proinflammatory cytokines, such as IL-1. c) IL-1 will then reach the brain via the humoral or neural pathway. d) In the brain, IL-1 can participate in the development of depression by for example (i) influencing the metabolism of neurotransmitters such as serotonin and (ii upregulate the hormone CRH. e) The activation of CRH in the paraventricular nucleus affects the release of ACTH and eventually the release of glucocorticoids (cortisol). f) Stressors also induce the release of acetylcholine trough the motor vagus nerve which result in withdrawal of inhibitory motor vagal input.

Chover-Gonzales et al. (1993) observed that IL-1 has an additive effect on CRH secretion from the PVN. As a response to acute stress, the concentration of IL-1 both peripherally and centrally is upregulated within 1 to 3 hours after the stress onset. Therefore, it is possible that IL-1 primes the HPA response. Another study by Gądek-Michalska et al. (2011) found that the administration of an IL-1 receptor antagonist diminished ACTH response to restraint stress. This indicates that IL-1 may partially mediate stress induced HPA activation. Besides cytokine interactions, cyclooxygenase-2 (COX-2) has also been shown to mediate TLR4 involvement in modulation of HPA activity, as described earlier (Liu et al., 2014). Pretreatment with specific inhibitors of COX-2 attenuates these responses (Dantzer, 2009).

It is also believed that TLR4 can influence HPA activity long after the stressor is resolved. Mouihate et al. (2010) found that a single lipopolysaccharide (LPS) challenge during early-life is sufficient to hypersensitize the CRH and ACTH response to both subsequent LPS and restraint stress when tested in adulthood. An early-life activation of TLR4 also results in an increase in anxiety behavior during adulthood (Sominsky et al., 2013). This suggests that TLR4 activity during developmentally sensitive periods may shape the HPA system, priming the system toward hyperreactivity, and may even be changing individual predisposition toward stress-related disorders (Liu et al., 2014).

Regulation of the Body Temperature by IL-1

Fever is a common symptom of infectious and inflammatory disease, and it is often accompanied by sickness feeling. It is defined as a regulated rise in the body temperature above normal daily fluctuations occurring in conjunction with an elevated thermoregulatory set point (Ogoina, 2011). It is well known that prostaglandin E_2 (PGE_2) can induce fever upon binding to its EP_3 receptor located in the preoptic hypothalamus (Blomqvist & Engblom, 2018).

The initiation, manifestation and regulation of the febrile response is dependent on the pyrogenic and anti-pyretic properties of various exogenous and endogenous substances (Ogoina, 2011). Pyrogens can directly and indirectly lead to fever, while cryogens prevent excessive temperature elevation. Pyrogens are classified into exogenous pyrogens, for example LPS, and endogenous pyrogens, for example IL-1. Cryogens include anti-inflammatory cytokines, hormones and other neuroendocrine products. Fever signals carried

by exogenous and endogenous pyrogens ultimately lead to a reset of the thermoregulatory circuitry via the humoral and neural pathways.

IL-1, and other pyrogenic cytokines, can induce fever through a direct and an indirect humoral pathway (Ogoina, 2011). In the indirect pathway, IL-1 acts outside the brain by binding to and activating cytokine receptors located on the fenestrated capillaries of the circumventricular organs (CVOs) leading to release of PGE_2. In the direct pathway, IL-1 can disrupt the blood brain barrier (BBB) gaining direct access to cytokine receptors expressed on vascular, glial and neuronal structures of the brain. Activation of these central receptors stimulates further synthesis of PGE_2 or promotes synthesis of more cytokines by the brain. Peripheral fever signals can also communicate with the CNS through peripheral nerves such as cutaneous sensory nerves and the vagus nerve.

The preoptic area (POA) of the anterior hypothalamus is considered as the major thermoregulatory center in the CNS (Ogoina, 2011). PGE_2 binds to EP_3 receptor in the POA and then activates thermal neurons in the anterior hypothalamus to a higher thermal balance point. Findings by Nakamura et al. (2002) suggest that the neurons in the POA in healthy animals provide a tonic inhibitory GABAergic input to thermogenic presympathetic neurons in the rostral medullary raphe nucleus (RMR) of the brain stem. The preoptic neurons are silenced when PGE_2 bind to their EP_3 receptors. This leads to a disinhibition of the presympathethic neurons. The neurons that innervate the spinal sympathetic preganglionic neurons (SPGNs) are termed presympathetic neurons. The SPGNs are the final common pathway for many reflexes important to homeostasis. A GABAergic neuron produces the amino acid gamma-aminobutyric acid (GABA) which acts as a neurotransmitter in the CNS and its natural function is to reduce the activity of the neurons to which it binds. The research on rats showed that EP_3 receptor expressing neurons in the POA project to the RMR and that a large majority of the EP_3 receptor expressing neurons in the POA co-express transcripts of the GABA-synthesizing enzyme GAD67. Recordings from cultured anterior hypothalamic neurons conducted by Tabarean et al. (2004) showed that PGE_2 decreased the firing rate in GABAergic neurons expressing EP_3 receptor. Preoptic neurons that are inhibited by PGE_2 have also been shown to be warm sensitive, and warm sensitive neurons are found to be GABAergic and to drive thermogenesis through descending projections. However, it is not clear to what extent the populations of warm sensitive and PGE_2-responsive neurons overlap (Tan et al., 2016).

Are there any Analogous Symptoms of Depression and Sickness Feeling?

Sickness feeling refers to the coordinated set of behavior changes that develop in individuals during the course of an infection (Roerink et al., 2017). It is characterized by hyperthermia, depressed mood, lethargy, sleep and appetite disturbances, reduced grooming and loss of interest in social interactions. Many of these effects can be attributed to an elevation of interleukin-1 (IL-1) in the brain.

Major depressive disorder (MDD) is a common disorder and poses one of the highest disease burdens worldwide (Liu et al., 2014). It is a serious mood disorder that causes severe symptoms which affect how you feel, think and handle daily activities. Some of the symptoms of this disorder are a persistent sad mood, moving or speaking more slowly, changes in appetite, lack of energy and disturbed sleep (National Institute of Mental Health, 2018). Many of those symptoms are similar to the ones seen in sickness feeling. Both conditions are characterized by lethargy and sleep- and appetite disturbances.

There are several forms of depression that are slightly different and can develop under unique circumstances. Some examples are persistent depressive disorder, postpartum depression, psychotic depression and seasonal affective disorder (National Institute of Mental Health, 2018). Persistent depressive disorder is a depressed mood that lasts for at least two years. Postpartum depression is major depression during pregnancy or after delivery. Psychotic depression occurs when a person has severe depression in addition to some form of psychosis. Seasonal affective disorder is characterized by the onset of depression during the winter months, when there is less natural sunlight. Depression can also be seen in relation to other brain diseases such as Parkinson's disease and Alzheimer's disease where it is a frequent comorbid condition (Liu et al., 2014). As described, depression is not a homogeneous disease and therefore it is unlikely that there is one specific factor that leads to depression. But because of the similarities in symptoms between sickness feeling and depression there is reason to believe that there might be a common molecular network playing a part in the onset and development of these conditions.

Increasing evidence suggests that inflammatory processes play an important role in the pathophysiology of depression (Farooq et al., 2017). Patients with MDD, who otherwise are medically healthy, have been found to have higher density of proinflammatory IL-1 both peripherally and in the CNS (Raison et al., 2006). These findings are amplified by Smith (1991) who suggested that excessive secretion of IL-1 is involved in the pathogenesis of

depression. The association between depression and inflammation are also described in patients with mild depressive symptoms such as fatigue, insomnia and anger. These patients are otherwise healthy, but there is evidence of inflammatory activation (Raison et al., 2006). Although there are studies that support the idea that inflammatory cytokines can contribute to depression, other studies do not find the association between inflammatory pathways and depression. Steptoe et al. (2003) did a study based on samples of healthy middle-aged men and women that failed to find an association between immune activation, inflammatory response and symptoms of depression. Another study reported that increased levels of cytokine production appeared only during the acute phase of depression (Seidel et al., 1995). Raison et al. (2006) found in some cases, the association have been attenuated or obviated when mediating factors such as body mass index, gender or personality, have been included in the analyses. These studies indicate that the role of the immune system influences in some cases of depression but not all.

HPA axis hyperactivity is a hallmark of major depression and can also be seen during an infection or inflammatory process when the individual is experiencing sickness feeling. Patients with MDD are found to have heightened levels of cortisol in the morning while the levels are possessing a flatter diurnal slope throughout the day (Dinan, 1994; Jarcho, Slavich, Tylova-Stein, Wolkowitz, & Burke, 2013). This indicates that a dysregulation in HPA activity is involved in the pathophysiology of depression.

MDD is also characterized by abnormalities in the central monoaminergic neurotransmitter system involving serotonin, noradrenaline and dopamine which leads to behavioral changes and alterations in neurohormonal pathways (Pasic et al., 2003; Raison et al., 2006). The most important neurotransmitter implicated in the pathophysiology of depression, is serotonin (Farooq et al., 2017). During an inflammatory process, the enzyme indoleamine-2,3 dioxygenase will be induced, and IL-1 will activate the enzyme which thereby converts tryptophan, a precursor of serotonin, to kynurenic acid and quionoacid. This results in decreased levels of tryptophan and therefore reduced synthesis of serotonin in the brain. This will reduce the level of serotonin in the synaptic cleft, which is often associated with depression. IL-1β also modulates the activity of the serotonin transporter, which function in serotonergic neurotransmission by reuptake of serotonin. In a study by Ramamoorthy et al. (1995), recombinant human IL-1β was injected into the hippocampus of a rat, resulting in sickness feeling, fever, increased levels of serotonergic transmission and activations of the HPA axis. The study showed that IL-1β is a potent stimulant of the serotonin transporters that

leads to decreased levels of serotonin in the synaptic cleft, which can be connected to mood disorders. IL-1 can also influence dopamine synthesis and dopamine transporters by affecting the synthesis of dopamine via oxidative stress and disruption of the enzyme tetrahydrobiopterin (BH4), which inhibits the conversion of phenylalanine to the precursor tyrosine and L-3,4-dihydroxyphenylalanine (Roerink et al., 2017).

Serotonin and dopamine neurotransmitters are primary targets for currently available antidepressant treatments, and selective serotonin and noradrenaline reuptake inhibitors (SSRI and SNRI respectively) are mostly utilized (Arroll et al., 2009; Pasic et al., 2003). SSRIs block the serotonergic reuptake in the presynaptic serotonergic terminal and thereby increase the serotonin level in the synaptic cleft. This will elevate mood and depression over time. Yet, the pharmacological treatments on the market today are inefficient. In order to gain one positive outcome, seven patients are required to be treated (Arroll et al., 2009). These facts implicate that the serotonergic pathway is only partly responsible for MDD, and other mechanisms must be involved (Liu et al., 2014).

The role of toll-like receptor 4 (TLR4) in depression has come to the forefront of research when it comes to neuroimmune signaling and depression (Liu et al., 2014). Kéri et al. (2014), did a study based on real-time quantitative PCR to measure TLR4 from peripheral blood mononuclear cells. The study showed that patients with MDD expressed higher density of TLR4. The expression was reduced after following treatments and the depressive symptoms were decreased as well. This indicates that TLR4 activity could directly be involved in the pathophysiology of depression. The TLR4 mediated innate immune process in the CNS is due to the resident glial cells, consisting of oligodendrocytes, astrocytes and microglia, and infiltrating peripheral immune cells (Liu et al., 2014). Astrocytes provide structural and trophic support to neurons. They can influence neurotransmission on synaptic levels and play a role in the aggregating neural responses by secreting substances, such as ATP and acetylcholine. Astrocytes can be involved in the pathophysiology of major depressive disorder (MDD) in the way that they play a role in serotonin neurotransmission. The serotonin transporters expressed on astrocytes increase the reuptake of serotonin, and thereby lower the serotonin level in the synaptic cleft. In contrast, microglia are the immunocompetent cells in the CNS. Their active role is to release cytokines, execute phagocytosis and remove debris during the inflammatory process (Liu et al., 2014). Microglia express TLR4 and are therefore responsive to pathogen-associated molecular patterns

(PAMPs). The TLR4 activation can activate microglia to another phenotype which display a more amoeboid morphology and thereby secrete cytokines that results in a proinflammatory response in the CNS. The changes in microglial reactivity states can be related to stress-induced depressive like behavior. It appears that microglia play an important role in central immune signaling including communication between immune and brain in MDD. However, this relation is not unidirectional and appears to be time dependent.

Sleep Deprivation Related to Interleukin-1 and Depression

Individual symptoms, especially sleep disturbance, have been reported to contribute to the association between inflammation and depression (Raison et al., 2006). Several studies have shown that during an infection the amount of sleep increases in mammals, due to the induction of proinflammatory IL-1 (Bollinger, Bollinger, Oster, & Solbach, 2010). IL-1β is proven to be a sleep regulatory substance (SRs) (Krueger, 2008). It has the capacity to enhance non-rapid eye movement sleep (NREMS) and contribute to the homeostatic regulation of slow-wave sleep (SWS) during an infectious challenge (Besedovsky, Lange, & Haack, 2019; Krueger, Walter, Dinarello, Wolff, & Chedid, 1984) . In addition, IL-1 inhibits wake-active neurons, while enhancing the firing rate of hypothalamic sleep-active neurons. IL-1R antagonist (IL-1Ra) was used to prevent the biological action of IL-1 which resulted in decreased physiological NREM sleep amount. When the access of IL-1 increased, NREM sleep amount was promoted and REM sleep amount was suppressed. However, it is still not clear if IL-1 plays a role in the physiological REM sleep regulation (Besedovsky et al., 2019). Even though sleep depends on other factors, such as time of the day, route and dose of administration, IL-1 is considered to be a substance that is involved in the homeostatic regulation of sleep (Krueger, 2008).

Sleep deprivation can result in issues due to neurobiology and neuropathology. During sleep deprivation IL-1 mRNA increases in the brain. Sleep deprivation and IL-1 enhances hypothalamic and cortical NFκB activation which acts as an enhancer element for a wide array of genes which includes other SRs and COX-2 (Krueger, 2008).

Discussion

There is a complex interaction between the central nervous system (CNS), the immune system and the endocrine system. Together, these mechanisms cooperate to take care of the body's homeostasis. The communication between the CNS and the peripheral immune system seems to be bidirectional (*Fig. 3*) (Leonard, 2018). It is connected via the common use of receptors, ligands and cell-to-cell communication (Juhász, 2008). The connection include communication via hormones, neurotransmitters, cytokines and nerves.

The immune system can communicate with the CNS through a humoral and a neural pathway, and vice versa. The vagus nerve is a part of the autonomic nervous system that innervates all organs, including the immune system (Juhász, 2008). The autonomic nervous system consists of two main systems: the sympathetic system that uses noradrenaline for neurotransmission and the parasympathetic system which consists of cholinergic nerves. The vagus nerve is a cholinergic nerve that is believed to be part of a cholinergic anti-inflammatory pathway. Cytokines can activate vagal nerve afferent fibers (Juhász, 2008). The vagal afferents project to the nucleus of the solitary tract (NTS) which activate the paraventricular nucleus (PVN) of the hypothalamus, one of the main regulatory centers of the hormonal system. This can cause an activation of the hypothalamus-pituitary-adrenal (HPA) axis. The humoral mechanisms are mediated by IL-1 crossing the blood-brain barrier (BBB) entering the cerebrospinal fluid (CSF) at circumventricular organs.

Brain-to-immune communication is the efferent part of the cholinergic anti-inflammatory pathway. The humoral pathway is the HPA axis, which reduces inflammation via releasing glucocorticoids and catecholamines. The vagus nerve innervates the thymus gland which plays an important role in the immune system because of its maturation of T-lymphocytes (Lea, 2006). Also T and B-cells have receptors for neurotransmitters that can be downregulated by acetylcholine (Juhász, 2008). The direct effect of the vagal efferents on immune cells has not been properly established yet, but it is believed that the vagal efferents are able to suppress the release of proinflammatory IL-1.

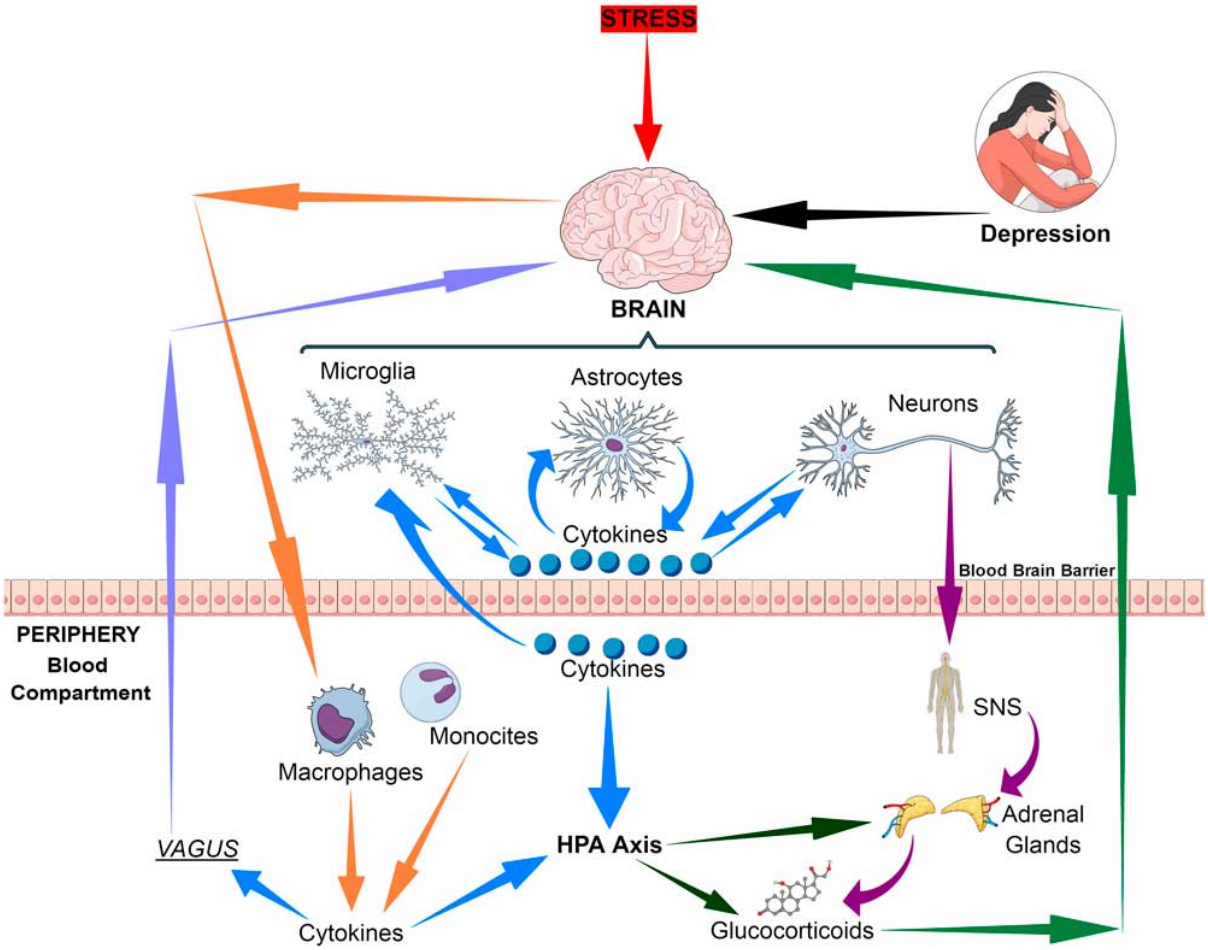

Figure 3 – The Bidirectional Connections Between Stress, the Central Nervous System and Inflammatory Cytokines (From Leonard (2018) with permission). Both neural and humoral processes are involved in the coordination of the physiological, immunological and behavioral responses following an inflammatory challenge. Afferent pathways are connected to the brain via cytokines and the vagus nerve, while the peripheral immune responses are modulated by the efferent pathways, through the hypothalamic–pituitary–adrenal (HPA) axis, the sympathetic nervous system (SNS) and the parasympathetic nervous systems.

The nervous-, immune- and endocrine system work closely together to protect the body and restore homeostasis. Sometimes when the stress response is prolonged it can lead to damage of the neuronal network and it becomes dysfunctional. This can be due to the activation of peripheral macrophages, the central microglia and hypercortisolemia caused by the activation of the HPA axis. When an infection arises in the brain, neuroinflammation, the innate cells in the brain (microglia, astrocytes and oligodendroglia) are activated to release cytokines in response to the inflammatory stimulus. When this infection becomes chronic, it is usually maladaptive and has a detrimental effect on the brain homeostasis. The activated microglia can be detrimental due to the prolonged neuroinflammatory changes. This process can be seen in for example depression.

Even though some studies do not find the association between inflammatory pathways and depression such as Steptoe et al. (2003) and Raison et al. (2006), most studies find alterations in immune function and increased levels of IL-1 in patients with MDD (Dantzer, 2009; Farooq et al., 2017; Liu et al., 2014; Pasic et al., 2003). The mechanisms leading to the development of depression and fever seems to be connected through a common molecular network initiated by the action of IL-1. This molecular network includes the activation of IL-1 family receptors expressed in the membrane of innate immune cells in the periphery and the CNS. The activation of IL-1R1 then leads to increasing levels of IL-1 and cyclooxygenase-2 (COX-2) which thereby induce the modulation of the HPA axis. COX-2 can further be connected to the development of fever because of its role in the production of PGE_2, while the hyperactivity of the HPA axis is able to cause heightened cortisol levels that can be linked to depression. IL-1 is also described to be involved in alterations of neurotransmitter function, especially decreased serotonin and dopamine levels, which is well known to induce depression (Pasic et al., 2003; Roerink et al., 2017). Sickness feeling can be connected to both depression and fever because of their similar symptoms, such as hypothermia, depressed mood, sleep and appetite disturbances and lethargy. Based on corresponding results of the role of IL-1 from several original- and review articles, there are reasons to believe that IL-1 links together sickness feeling, fever and depression.

References

Arroll, B., Elley, C. R., Fishman, T., Goodyearsmith, F. A., Kenealy, T., Blashki, G., . . . Macgillivray, S. (2009). Antidepressants versus placebo for depression in primary care. *Cochrane Depression, Anxiety and Neurosis Group*(3). doi:10.1002/14651858.CD007954

Atkins, E. (1982). Fever: its history, cause, and function. *The Yale journal of biology and medicine, 55*(3-4), 283-289.

Besedovsky, L., Lange, T., & Haack, M. (2019). The Sleep-Immune Crosstalk in Health and Disease. *Physiological reviews, 99*(3), 1325-1380. doi:10.1152/physrev.00010.2018

Bishop, M. L., Fody, E. P., & Schoeff, L. E. (2018). *Clinical chemistry : principles, techniques, and correlations* (8 ed.). Philadelphia: Wolters Kluwer.

Blomqvist, A., & Engblom, D. (2018). Neural Mechanisms of Inflammation-Induced Fever. In (Vol. 24, pp. 381-399). Los Angeles, CA.

Bollinger, T., Bollinger, A., Oster, H., & Solbach, W. (2010). Sleep, Immunity, and Circadian Clocks: A Mechanistic Model. *Gerontology, 56*(6), 574-580. doi:10.1159/000281827

Chover-Gonzalez, A. J., Harbuz, M. S., & Lightmann, S. L. (1993). Effect of adrenalectomy and stress on interleukin-1β-mediated activation of hypothalamic corticotropin-releasing factor mRNA. *Journal of Neuroimmunology, 42*(2), 155-160. doi:10.1016/0165-5728(93)90005-J

Dahl, H. A., & Rinvik, E. (2010). *Menneskets funksjonelle anatomi : med hovedvekt på bevegelsesapparatet* (3 ed.). Oslo: Cappelen akademisk.

Dantzer, R. (2009). Cytokine, Sickness Behavior, and Depression. *Immunology and Allergy Clinics of North America, 29*(2), 247-264. doi:10.1016/j.iac.2009.02.002

Dinan, T. G. (1994). Glucocorticoids and the genesis of depressive illness. A psychobiological model. *The British journal of psychiatry : the journal of mental science, 164*(3), 365. doi:10.1192/bjp.164.3.365

Dinarello, C. A. (2015). The history of fever, leukocytic pyrogen and interleukin-1. *Temperature, 2*(1), 8-16. doi:10.1080/23328940.2015.1017086

Ericsson, A., Liu, C., Hart, R. P., & Sawchenko, P. E. (1995). Type 1 interleukin‐1 receptor in the rat brain: Distribution, regulation, and relationship to sites of IL-1-induced cellular activation. *Journal of Comparative Neurology, 361*(4), 681-698. doi:10.1002/cne.903610410

Farooq, R. K., Asghar, K., Kanwal, S., & Zulqernain, A. (2017). Role of inflammatory cytokines in depression: Focus on interleukin-1beta. *Biomed Rep, 6*(1), 15-20. doi:10.3892/br.2016.807

Felten, D. L. (2003). *Netter's atlas of human neuroscience*. Teterboro, N.J: Icon Learning Systems.

Gądek-Michalska, A., Tadeusz, J., Rachwalska, P., Spyrka, J., & Bugajski, J. (2011). Effect of prior stress on interleukin-1β and HPA axis responses to acute stress. *Pharmacological Reports, 63*(6), 1393-1403. doi:10.1016/S1734-1140(11)70703-4

Harden, L. M., Kent, S., Pittman, Q. J., & Roth, J. (2015). Fever and sickness behavior: Friend or foe? *Brain Behavior and Immunity, 50*, 322-333. doi:10.1016/j.bbi.2015.07.012

Jarcho, M. R., Slavich, G. M., Tylova-Stein, H., Wolkowitz, O. M., & Burke, H. M. (2013). Dysregulated diurnal cortisol pattern is associated with glucocorticoid resistance in women with major depressive disorder. *Biological Psychology, 93*(1), 150-158. doi:10.1016/j.biopsycho.2013.01.018

Juhász, G. (2008). Neuroimmune Cross Talk. In A. Lajtha, A. Galoyan, & H. O. Besedovsky (Eds.), *Handbook of Neurochemistry and Molecular Neurobiology: Neuroimmunology* (pp. 293-307). Boston, MA: Springer US.

Katsuura, G., Gottschall, P. E., & Arimura, A. (1988). Identification of a high-affinity receptor for interleukin-1 beta in rat brain. *Biochemical and biophysical research communications., 156*(1), 61-67. doi:10.1016/s0006-291x(88)80805-2

Krueger, J. M. (2008). The Role of Cytokines in Sleep Regulation. *Current Pharmaceutical Design, 14*(32), 3408-3416. doi:10.2174/138161208786549281

Krueger, J. M., Walter, J., Dinarello, C. A., Wolff, S. M., & Chedid, L. (1984). Sleep-promoting effects of endogenous pyrogen (interleukin-1). *The American journal of physiology, 246*(6 Pt 2), R994-R999.

Kéri, S., Szabó, C., & Kelemen, O. (2014). Expression of Toll-Like Receptors in peripheral blood mononuclear cells and response to cognitive-behavioral therapy in major depressive disorder. *Brain Behavior and Immunity, 40*, 235-243. doi:10.1016/j.bbi.2014.03.020

Lawrence, T. (2009). The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harbor perspectives in biology, 1*(6), a001651-a001651. doi:10.1101/cshperspect.a001651

Lea, T. (2006). *Immunologi og immunologiske teknikker* (3 ed.). Bergen: Fagbokforlaget.

Leonard, B. E. (2018). Inflammation and depression: a causal or coincidental link to the pathophysiology? *Acta neuropsychiatrica, 30*(1), 1-16. doi:10.1017/neu.2016.69

Liu, J., Buisman-Pijlman, F., & Hutchinson, M. (2014). Toll-like receptor 4: innate immune regulator of neuroimmune and neuroendocrine interactions in stress and major depressive disorder. *Frontiers in Neuroscience, 8*. doi:10.3389/fnins.2014.00309

Masanori, Y., Yasuhisa, T., Asami, E., Satoshi, K., Yukiharu, M., Masayuki, N., . . . Yosky, K. (2014). Brain interleukin-1β and the intrinsic receptor antagonist control peripheral Toll-like receptor 3-mediated suppression of spontaneous activity in rats. *PLoS ONE, 9*(3), e90950. doi:10.1371/journal.pone.0090950

Mathers, C. D., & Loncar, D. (2006). Projections of Global Mortality and Burden of Disease from 2002 to 2030 (Projections of Global Mortality). *PLoS Medicine, 3*(11), e442. doi:10.1371/journal.pmed.0030442

Mouihate, A., Galic, M. A., Ellis, S. L., Spencer, S. J., Tsutsui, S., & Pittman, Q. J. (2010). Early life activation of toll-like receptor 4 reprograms neural anti-inflammatory pathways. *The Journal*

of neuroscience : the official journal of the Society for Neuroscience, 30(23), 7975. doi:10.1523/JNEUROSCI.6078-09.2010

Nakamura, K., Matsumura, K., Kaneko, T., Kobayashi, S., Katoh, H., & Negishi, M. (2002). The rostral raphe pallidus nucleus mediates pyrogenic transmission from the preoptic area. *The journal of neuroscience : the official journal of the Society for Neuroscience., 22*(11), 4600-4610. doi:20026439

National Institute of Mental Health. (2018, February). Depression. Retrieved from https://www.nimh.nih.gov/health/topics/depression/index.shtml

Ogoina, D. (2011). Fever, fever patterns and diseases called 'fever' – A review. *Journal of Infection and Public Health, 4*(3), 108-124. doi:10.1016/j.jiph.2011.05.002

Parnet, P., Kelley, K. W., Bluthé, R.-M., & Dantzer, R. (2002). Expression and regulation of interleukin-1 receptors in the brain. Role in cytokines-induced sickness behavior. In (Vol. 125, pp. 5-14).

Pasic, C. J., Levy, D. W., & Sullivan, D. M. (2003). Cytokines in Depression and Heart Failure. *Psychosomatic Medicine, 65*(2), 181-193. doi:10.1097/01.PSY.0000058372.50240.38

Raison, C. L., Capuron, L., & Miller, A. H. (2006). Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends in Immunology, 27*(1), 24-31. doi:10.1016/j.it.2005.11.006

Ramamoorthy, S., Ramamoorthy, J. D., Prasad, P. D., Bhat, G. K., Mahesh, V. B., Leibach, F. H., & Ganapathy, V. (1995). Regulation of the Human Serotonin Transporter by Interleukin-1β. *Biochemical and Biophysical Research Communications, 216*(2), 560-567. doi:10.1006/bbrc.1995.2659

Roerink, M. E., van der Schaaf, M. E., Dinarello, C. A., Knoop, H., & van der Meer, J. W. (2017). Interleukin-1 as a mediator of fatigue in disease: a narrative review. *J Neuroinflammation, 14*(1), 16. doi:10.1186/s12974-017-0796-7

Sand, O., Sjaastad, Ø. v., Haug, E., & Bjålie, J. G. (2006). *Menneskekroppen : fysiologi og anatomi* (2 ed.). Oslo: Gyldendal akademisk.

Seidel, A., Arolt, V., Hunstiger, M., Rink, L., Behnisch, A., & Kirchner, H. (1995). Cytokine Production and Serum Proteins in Depression. *Scandinavian Journal of Immunology, 41*(6), 534-538. doi:10.1111/j.1365-3083.1995.tb03604.x

Smith, R. S. (1991). The macrophage theory of depression. *Medical Hypotheses, 36*(2), 178-178. doi:10.1016/0306-9877(91)90266-2

Sominsky, L., Fuller, E. A., Bondarenko, E., Ong, L. K., Averell, L., Nalivaiko, E., . . . Hodgson, D. M. (2013). Functional Programming of the Autonomic Nervous System by Early Life Immune Exposure: Implications for Anxiety.(Research Article). *PLoS ONE, 8*(3), e57700. doi:10.1371/journal.pone.0057700

Steptoe, A., Kunz-Ebrecht, S. R., & Owen, N. (2003). Lack of association between depressive symptoms and markers of immune and vascular inflammation in middle-aged men and women. *Psychol. Med., 33*(4), 667-674. doi:10.1017/S0033291702007250

Tabarean, I. V., Behrens, M. M., Bartfai, T., & Korn, H. (2004). Prostaglandin E2-increased thermosensitivity of anterior hypothalamic neurons is associated with depressed inhibition. *Proceedings of the National Academy of Sciences of the United States of America, 101*(8), 2590. doi:10.1073/pnas.0308718101

Tan, C. L., Cooke, E. K., Leib, D. E., Lin, Y.-C., Daly, G. E., Zimmerman, C. A., & Knight, Z. A. (2016). Warm-Sensitive Neurons that Control Body Temperature. *Cell, 167*(1), 47-59.e15. doi:10.1016/j.cell.2016.08.028

Definitions

Adrenocorticotropic hormone (ACTH): A hormone that is produced by the anterior lobe of the pituitary gland and that stimulates the secretion of cortisone, aldosterone and other hormones by the adrenal cortex.

Afferent: Relating to a nerve that carries sensory information toward the central nervous system.

Amygdala: An almond-shaped brain nucleus at the front of the temporal lobe parts of the brain that affect emotions, especially fear and pleasure.

Anterior: Located in front.

Brain parenchyma: Refers to the functional tissue in the brain that is made up of the two

Cerebellum: Part of the brain that plays an important role of motor control.

Cholinergic: Relating to nerve cells or fibers that use acetylcholine as their neurotransmitter.

Choroid plexus: Specialized cells located in the ependyma of the circumventricular organs (CVOs). It consists of many small blood vessels that is responsible for the production of the cerebrospinal fluid (CSF).

Circumventricular organs (CVOs): Structures in the brain that are characterized by their extensive vasculature and lack of a normal blood brain barrier (BBB). The CVOs allow for the linkage between the central nervous system and peripheral blood flow.

Corticotrophin-releasing hormone (CRH): A peptide hormone involved in the stress response. Its main function is the stimulation of the pituitary synthesis of adrenocorticotropic hormone (ACTH), as part of the HPA axis.

Efferent: Relating to a nerve that carries motor impulses from the central nervous system to the muscles.

Ependyma: The membrane lining the cerebral ventricles and the central canal of the spine.

GABAergic: Neurons that produce and release GABA as their neurotransmitter.

Gamma-aminobutyric acid (GABA): An inhibitory neurotransmitter in the central nervous system produced by GABAergic neurons. Its principal role is to reduce neuron excitability.

Hippocampus: A limbic system structure located in the temporal lobe of the cerebral cortex. The function is processing short-term memory, encoding explicit memories for long-term storage, controlling autonomic functions, emotional expression, subjective experience, behavioral expression of affect.

Hypothalamic-pituitary-adrenal (HPA) axis: The combined system of neuroendocrine units that in a negative feedback network regulate the adrenal gland's hormonal activities.

In situ hybridization: A type of hybridization that uses a labeled complementary DNA, RNA or modified nucleic acids strand to localize a specific DNA or RNA sequence in a portion or section of tissue.

Interleukin-1 receptor-associated kinases 1 and 2 (IRAK1 and IRAK2): These are essential components of the Interleukin-1 receptor signaling pathway and some Toll-like receptor signaling pathways. They are membrane proximal putative serine-threonine kinases.

Limbic system: The limbic system is located in the forebrain. It is associated with olfaction, autonomic functions and certain aspects of emotion and behavior.

Monoaminergic: Referring to neurons that secrete the monoamine neurotransmitters dopamine, noradrenaline and serotonin.

Myeloid differentiation primary response 88 (MyD88): An adaptor protein for inflammatory signaling pathways downstream of members of the Toll-like receptor (TLR) and interleukin-1 (IL-1) receptor families. MyD88 links IL-1 receptor (IL-1R) or TLR family members to IL-1R-associated kinase (IRAK).

Non-rapid eye movement sleep (**NREMS):** Sleep during which non–rapid eye movements occur. Sleep is broken down into five phases: wake, N1, N2, N3 and R. Stages N1-N3 are considered as NREMS, each progressively going into deeper sleep.

Nuclear factor kappa-light-chain-enhancer and activated B-cells (NF*κ*B): A gene transcription regulator (protein complex) that controls transcription of DNA, cytokine production, upregulation of COX-2 and cell survival.

Nucleus of the solitary tract (NTS): The only brainstem nucleus of the visceral sensory column. It runs the length of the caudal hindbrain in the lateral subventricular gray matter alongside the solitary tract.

Perivascular: This is a fluid-filled space surrounding several blood vessels in for example the brain. It is considered to have an immunological function.

Posterior: Located at the back or behind for example another organ in the body.

Preoptic area (POA): Located at the anterior hypothalamus. This area is considered as the major thermoregulatory center in the CNS.

Prostaglandin E_2-group (PGE2): It is a small bioactive lipid working as the main mediator of cytokine-induced fever and activation of the HPA axis.

Radioiodinated: Treated or combined with radioiodine, which are any radioactive isotope of iodine.

Rapid eye movement (REM) sleep: Characterized by rapid eye movement hence its name. Vivid dreams usually happen during REM sleep.

Riboprobe: A short, radioactively labeled nucleotide sequence used in molecular hybridization to identify specific RNA or DNA sequences.

Rostral medullary raphe nucleus (RMR): A moderate-size cluster of serotonergic neurons found in the brain stem.

Selective noradrenaline reuptake inhibitors (SNRIs): Any class of drugs that selectively inhibit the reuptake of the neurotransmitter noradrenaline in the central nervous system. They are used primarily in the treatment of depression.

Selective serotonergic reuptake inhibitors (SSRIs): Any class of drugs that selectively inhibit the reuptake of serotonin by neurons of the central nervous system. They are primarily used in the treatment of depression and obsessive-compulsive disorder.

Slow-wave sleep (SWS): A recurrent period of deep sleep distinguished by the presence of slow brain waves and by very little dreaming.

Spinal sympathetic preganglionic neurons (SPGNs): Neurons located within the spinal cord whose axons traverse the ventral horn to exit in ventral roots where they form synapses onto postganglionic neurons. These neurons are the last point at which the central nervous system (CNS) can exert an effect to enable changes in the sympathetic outflow.

Tetrahydrobiopterin (BH4): A cofactor used in the degradation of the amino acid phenylalanine and in the biosynthesis of the neurotransmitters serotonin, melatonin, dopamine, noradrenaline, adrenaline**.**

The vagus nerve: A nerve that supplies nerve fibers to the pharynx (throat), larynx (voice box), trachea (windpipe), lungs, heart, esophagus and intestinal tract, as far as the transverse portion of the colon. The vagus nerve also brings sensory information back to the brain.

Tumor-necrosis factor (TNF): A multifunctional cytokine secreted by inflammatory cells that plays important roles in diverse cellular events such as cell survival, proliferation, differentiation and death.