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# Coupled cell networks are target cells of inflammation, which can spread between different body organs and develop into systemic chronic inflammation

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## Abstract

Several organs in the body comprise cells coupled into networks. These cells have in common that they are excitable but do not express action potentials. Furthermore, they are equipped with $Ca^{2+}$ signaling systems, which can be intercellular and/or extracellular. The transport of small molecules between the cells occurs through gap junctions comprising connexin 43. Examples of cells coupled into networks include astrocytes, keratinocytes, chondrocytes, synovial fibroblasts, osteoblasts, connective tissue cells, cardiac and corneal fibroblasts, myofibroblasts, hepatocytes, and different types of glandular cells. These cells are targets for inflammation, which can be initiated after injury or in disease. If the inflammation reaches the CNS, it develops into neuroinflammation and can be of importance in the development of systemic chronic inflammation, which can manifest as pain and result in changes in the expression and structure of cellular components. Biochemical parameters of importance for cellular functions are described in this review.

**Keywords:** Coupled cell networks, Inflammation, Pain spreading, $Ca^{2+}$ signaling, Connexin 43, Gap junctions

## Introduction

### Inflammation and neuroinflammation

In conditions that lead to inflammation, changes in several cellular parameters of coupled cell networks occur throughout many organs in the body. Inflammation is a physiological response to injury that is designed to remove dangerous stimuli, kill bacteria and viruses, remove cell debris, and initiate healing. It can persist or become exaggerated and may cause undesirable negative effects. Inflammation can be induced by several substances produced or released by tissues or by environmental factors, including circulating glucose, gut microflora, interleukins, endotoxins or other toxins, all of which have an impact on immune receptor expression. The underlying molecular processes are just beginning to be elucidated.

When an injury occurs in peripheral tissue, pro-inflammatory mediators are released into the bloodstream, and white blood cells are attracted to the injury site. The endothelium lining the blood vessels becomes permeable, allowing leukocytes to migrate from the blood vessels to the injury site [1, 2]. The pro-inflammatory mediators released can increase the permeability of the blood–brain barrier (BBB), leading to the passage of blood cells into the central nervous system (CNS) [3, 4]. This process is known as neuroinflammation [5]. These blood cells are transformed into reactive microglia, which produce pro-inflammatory cytokines and activate astrocytes. This combined response causes a change in astrocyte network signaling, which is involved in monitoring neuronal signaling as well as rebuilding synapses [6].

Neuroinflammation can also be initiated when a local peripheral injury gives rise to inflammatory activation in the CNS at the site of the damaged or affected nerve(s) [7–9]. The inflammatory cascade is activated, and immunocompetent cells migrate to the site of injury. Such cells can be mast cells, which are capable of migrating

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across the BBB in situations where the barrier is compromised as a result of CNS pathology [10]. Pericytes in the microvessels respond to immune activation and may play an important role in communicating inflammatory signals [11]. Myofibroblasts, developed from fibroblasts and maybe also from pericytes, are considered to be the dominant collagen-producing cells and are activated when structural and functional defects occur [12]. As a result, the subsequent neuroinflammatory environment causes the activation of glial cells located in the dorsal horn of the spinal cord. Macrophages infiltrate the injured nerve and cause an inflammatory reaction in the neurons [13], which leads to microglial activation in the CNS and pro-inflammatory cytokine release. These cytokines then activate and alter astrocyte function [9, 14]. Once the astrocytes and microglia have been activated, they participate in the development, spread, and potentiation of neuroinflammation [15, 16], resulting in low-grade inflammation [7] along the pain pathways from the periphery to the spinal cord, extending up to the thalamus and farther onto the parietal cortex.

If this dysfunction persists for a long time, it can lead to pathogenic chronic neuroinflammation and can transition into long-term pain [8, 9, 17]. When an inflammatory response is activated throughout the body, the event can affect non-lesioned structures on both the ipsilateral and contralateral sides [18].

### Coupled cell networks

Similarities exist between different types of coupled cell networks in different body organs with respect to several cellular parameters. Examples of cells coupled into networks include astrocytes, keratinocytes, chondrocytes, synovial fibroblasts, osteoblasts, connective tissue cells, cardiac and corneal fibroblasts, myofibroblasts, hepatocytes, and different types of glandular cells (Fig. 1). Intercellular communication gives tissues the ability to coordinate many cellular functions such as the regulation of cell volume, intracellular ionic composition, and cell metabolism. Characteristics such as their passive electrical properties not only provide the framework and metabolic support for different organs but also contribute to their computational power and behavioral output. These properties enable more active functions and are endowed through $Ca^{2+}$-based excitability [19]. Intracellular $Ca^{2+}$ changes are important due to their influence on many cell functions, including matrix synthesis and degradation [20]. An increase in cytosolic $Ca^{2+}$ levels can lead to the release of signaling molecules such as transmitters, cytokines, prostaglandins, proteins, and peptides via regulated exocytosis [21]. The dynamic components of exocytosis include the vesicular-plasma membrane secretory machinery and vesicular traffic, which is governed by general cytoskeletal elements [22]. For this machinery to work, intercellular structures called gap junctions, which directly connect the interior of adjacent cells through a pathway not open to the extracellular space, appear necessary [23]. Gap junction channels comprise two hemichannels, called connexons, one of which is provided by each of the joined cells. These channels select for the direct exchange of ions, metabolites, and small molecules such as $Ca^{2+}$, adenosine triphosphate (ATP), nicotinamide adenine dinucleotide ($NAD^+$), glutamate, prostaglandins, and glutathione, which are less than 1.5 kDa in size, between contiguous cells [24]. Connexin 43 (Cx43) is the primary gap junction protein [25]. Cytoskeletal reorganization is pivotal event in all of these processes; dynamic remodeling of the actin cytoskeleton plays an essential role in cell migration and proliferation. Actin appears in two forms, globular actin (G-actin) and filamentous actin (F-actin), and the transition between these two forms is a dynamic process driven by polymerization and depolymerization [26] (Fig. 2).

## Coupled cell networks in different body organs

### Astrocytes

The well-studied cells coupled into networks are astrocytes in the CNS [6, 21]. Astrocytes in networks positioned between the vasculature and synapses monitor neuronal signaling and synapse rebuilding [6]. Astrocytes express nearly the same repertoire of receptors and ion channels as neurons, regulate synaptic transmission via bidirectional communication with neurons, and release gliotransmitters and other factors such as cytokines, fatty acid metabolites, and free radicals [27, 28].

Because they do not communicate via action potentials, astrocytes are not electrically active; however, they display a form of excitability that manifests as an increased intracellular $Ca^{2+}$ concentration. Stimuli such as transmitters released from neurons and glial cells can evoke $Ca^{2+}$ elevation in single astrocytes, which passes to adjacent astrocytes and leads to a $Ca^{2+}$ wave that can propagate over long distances, albeit much more slowly than the propagation of action potentials in neurons [19, 29, 30].

Incoming stimuli activate G protein-coupled receptors that hydrolyze phosphatidylinositol 4,5-bisphosphate ($PIP_2$) and cause the release of inositol 1,4,5-trisphosphate ($IP_3$) into the cytosol. $IP_3$ receptors located on the endoplasmic reticulum respond to this elevation of $IP_3$ by releasing $Ca^{2+}$. Cytosolic $Ca^{2+}$ plays a key role as a second messenger; thus, the control of $Ca^{2+}$ signals is critical. This control involves coordinating $Ca^{2+}$ entry across the plasma membrane, $Ca^{2+}$ release from the endoplasmic reticulum, endoplasmic reticulum store refilling, and $Ca^{2+}$ extrusion across the plasma membrane [31]. The $Na^+$-$Ca^{2+}$ exchanger, a $Ca^{2+}$ transporter that controls the intracellular $Ca^{2+}$ concentration, is driven by the $Na^+$ electrochemical gradient across the plasma membrane.This $Na^+$ pump, the $Na^+/K^+$-ATPase, indirectly modulates $Ca^{2+}$ signaling [32],

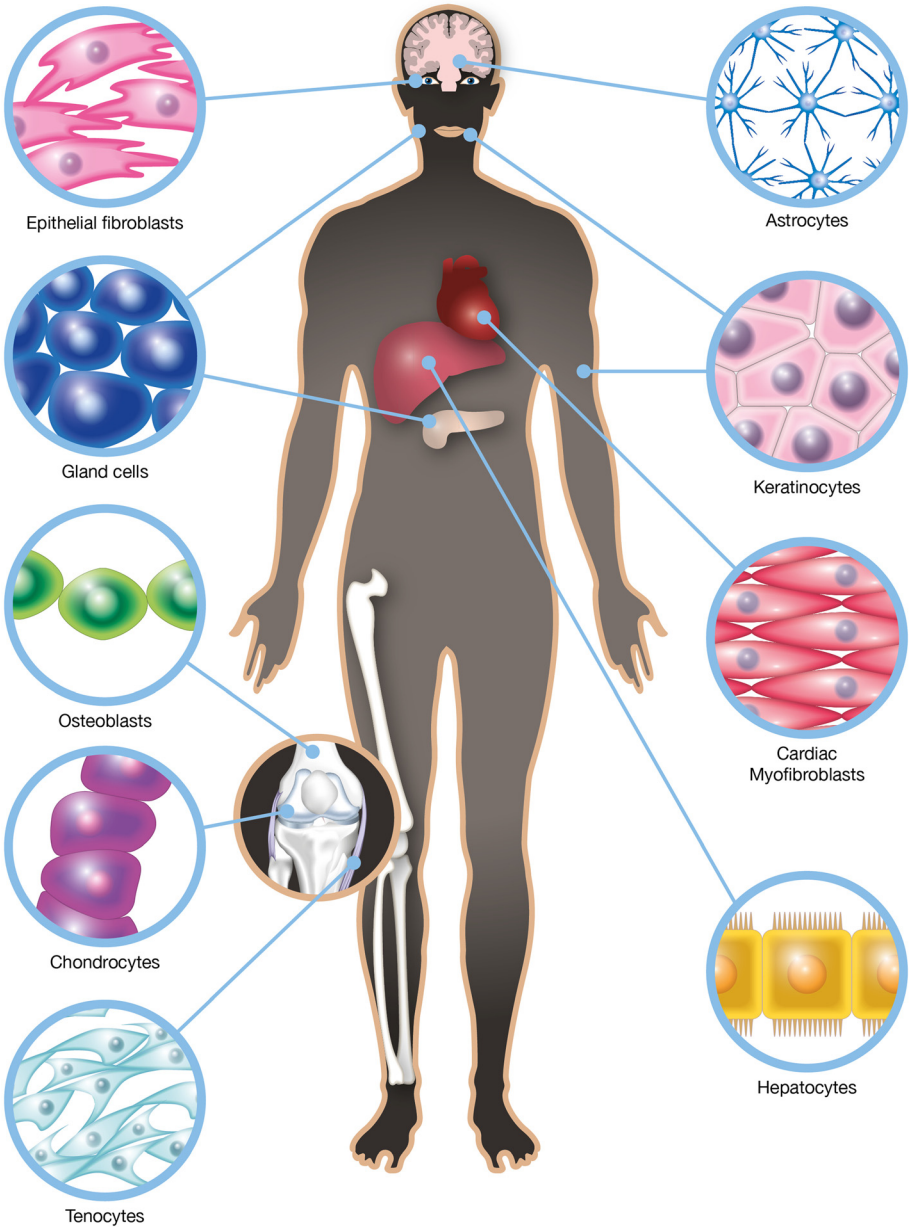


**Fig. 1** Schematic illustration highlighting the different organs in the body that comprise cells coupled into networks. These cells are excitable but do not express action potentials. They are equipped with $Ca^{2+}$ signaling systems, and the transport of small molecules between the cells occurs through gap junctions. Examples of cells coupled into networks are astrocytes in the brain, keratinocytes in the skin and buccal membranes, chondrocytes in the articular cartilage, osteoblasts in bone, connective tissue cells such as epithelial cells in the cornea and tenocytes in the ligaments, cardiac fibroblasts in the heart, hepatocytes in the liver, and different types of glandular cells throughout the body. The illustration was created by Pontus Andersson, ArtProduction, Gothenburg, Sweden

and inflammatory stimuli influence $Ca^{2+}$ homeostasis in astrocyte networks [33–35].

Astrocytes are directly connected to adjacent cells by gap junctions, and Cx43 is the primary gap junction protein [25]. Astrocytes also express hemichannels that open exteriorly; Cx43 appears to also be the main Cx found in these hemichannels [24]. Astrocytes in most parts of the CNS use two types of $Ca^{2+}$ communication: intercellular communication through gap junctions and extracellular communication through the diffusion of ATP, which then binds to purinoceptors. Both inter- and extracellular $Ca^{2+}$ communication occur in many parts of the cerebrum [19, 36]. In the retina, intercellular communication occurs through astrocytes, but extracellular communication occurs between astrocytes and Müller cells [37].

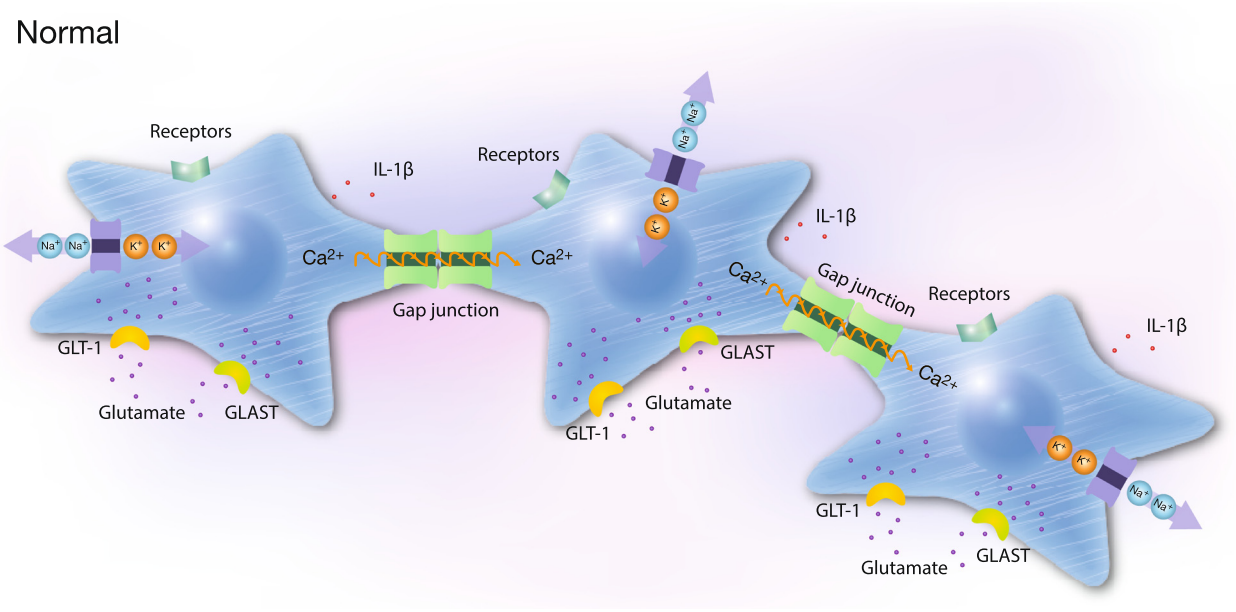


**Fig. 2** Schematic illustration of three cells coupled in networks under physiological conditions. The cells are coupled by gap junctions to form extensive cellular networks. Receptors, ion pumps, glutamate transporters, actin filaments (white bands), cytokine release and $Ca^{2+}$ signaling are shown. Cytosolic $Ca^{2+}$ plays key roles as a second messenger and a sensor of $Ca^{2+}$-dynamics in cell networks and detects changes in the microenvironment. Two communication pathways exist: the major intercellular via gap junctions comprising Cx43. Receptors on the surface of cells are coupled to G proteins and release $Ca^{2+}$ from the endoplasmic reticulum via PLC and $IP_3$. $Ca^{2+}$ elevations are followed by the propagation of intercellular $Ca^{2+}$ waves through the gap junctions, which are also permeable to molecules less than 1.5 kDa such as $Ca^{2+}$, $IP_3$, and cAMP. The minor extracellular pathway involves ATP release through hemichannels. The illustration was created by Pontus Andersson, ArtProduction, Gothenburg, Sweden

The cytoskeleton is important for controlling plasma membrane microdomains and the endoplasmic reticulum complex. The adaptor protein ankyrin B is associated with the $Na^+$ pump as well as with endoplasmic reticulum proteins such as $IP_3$. The primary cytoplasmic matrix proteins spectrin and actin are attached to ankyrin B. An intact cytoskeleton is required for astrocytic $Ca^{2+}$ wave propagation [36], and cytoskeletal disruption abolishes $Ca^{2+}$ oscillations by changing the balance of the $Ca^{2+}$ regulatory processes [38].

Astrocytes contribute to the homeostasis and regulation of extracellular glutamate levels. The glutamate-glutamine cycle is a well-known process through which glutamine is released from astrocytes and taken up by glutamatergic or γ-aminobutyric acid (GABA)ergic neurons. Glutamine is then converted to glutamate in neurons and released into the synaptic space. The majority of the released glutamate is taken up by astrocytes through the glutamate transporters; glutamate/aspartate transporter (GLAST/excitatory amino acid transporter 1, EAAT1), and glial glutamate transporter-1 (GLT-1/EAAT2) and then metabolized by glutamine synthetase to glutamine [39, 40].

### Keratinocytes

The epidermis is a dynamic, stratified structure formed by continually proliferating and differentiating keratinocytes that surround the sensory nerve endings of several C- and Aδ-fiber subtypes. The skin and buccal membrane primarily comprise keratinocytes, the epidermis. The cells are connected by well-developed intercellular junctions such as gap junctions. Within these gap junctions, Cx43 is associated with the regulation of cell proliferation and mediates forms of intercellular communication in which ions and small molecules are allowed to pass from one cell to another. Cx43 is primarily localized to the lower epithelial layer, the stratum basale and stratum spinosum [41]. Cx43 degradation is thought to play a role in the differentiation of the gingival epithelium. Properly regulated gap junctions appear to be essential for efficient wound healing and for protection against skin diseases. Human epidermal keratinocytes use intercellular $Ca^{2+}$ signaling. G protein-coupled receptors, which activate phospholipase C (PLC) and convert $PIP_2$ into diacylglycerol and $IP_3$, trigger the release of $Ca^{2+}$ from intracellular $Ca^{2+}$ stores [42, 43].

In response to stress, injury or even chronic pain, keratinocytes can release ATP through hemichannels, resulting in Cx43 upregulation. Metabotropic purinergic ($P2Y_2$) receptors are then activated, resulting in increased intracellular $Ca^{2+}$ [44]. ATP release is an important signal for epidermal homeostasis and influences keratinocyte proliferation and differentiation [45]. Glutamate-mediated signaling is observed in keratinocytes in the epidermis, and

different classes of glutamate receptors, including N-methyl-D-aspartate receptor (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) and metabotropic glutamate receptors, as well as transporters such as EAAT1 have been identified in the basal layer. Additionally, GLT-1 has been found in the suprabasal layer [46].

### Chondrocytes

Chondrocytes are connected to each other via cell-to-cell interactions and form functional gap junctions that express Cx43 [47, 48]. They can sustain the propagation of intercellular $Ca^{2+}$ waves in rabbits [47], humans, and equines [49, 50] and can also form hemichannels that exchange signals within the extracellular space [51] (Skiöldebrand et al., unpublished). Articular chondrocytes accumulate intracellular $IP_3$ following mechanical stimulation, causing the diffusion of $IP_3$ into adjacent cells through gap junctions and amplification of the response. In adult articular cartilage, chondrocytes exist as individual cells embedded in the extracellular matrix, and gap junctions are mainly expressed by the flattened chondrocytes facing the outer cartilage layer where intercellular communication occurs [52]. The role of Cx43 in chondrocytes has not been extensively studied, but Cx43 is required for the differentiation and metabolic homeostasis of the extracellular matrix [48]. Cx43 also functions as a hemichannel to release ATP and $NAD^+$. Chondrocytes express purinergic receptors such as P2-purinoceptors that induce intracellular $Ca^{2+}$ responses. These intracellular $Ca^{2+}$ responses are increased following stimulation with IL-1 [53]. Glutamate and substance P have been identified in human articular chondrocytes. Neurokinin 1 (NK1) and glutamate receptors are also expressed, as well as both metabotropic and ionotropic glutamate receptors and the glutamate transporters GLT-1 and GLAST [54].

### Bone cells

Gap junctional communication plays a critical role in the coordination of bone remodeling. The bone-forming cells osteoblasts and osteocytes primarily express Cx43 but also express Cx45 and Cx46, which form functional gap junctions [55]. Cx43 expression increases during differentiation, and inhibition of this communication leads to retardation of the differentiation process, resulting in a reduced ability to form mineralized extracellular matrix. Through mechanical manipulation, the osteoblasts, which are non-excitable, produce synchronized $Ca^{2+}$ waves, which involve the release of $IP_3$-sensitive intracellular $Ca^{2+}$ stores. These waves occur either via gap junction-mediated intercellular $Ca^{2+}$ signaling or as a result of the autocrine activity of released ATP, which stimulates P2 purinoceptors. The P2Y class comprises G protein-coupled receptors that activate PLC, resulting in $IP_3$ generation and intracellular $Ca^{2+}$ store release in human osteoblasts [56]. Hemichannels have also been reported in osteoblasts [55].

### Connective tissue cells

Gap junctions are found in tendons, ligaments, synovium (within the synovial membrane), and corneal stroma because the cells of these tissues are coupled to form networks. Two adjacent cells join through Cx43, allowing direct cell-to-cell communication via $Ca^{2+}$ signaling. In osteoarthritis, the synovial fibroblasts produce pro-inflammatory cytokines and catabolic proteases, leading to degradation of the extracellular matrix. The role of Cx43 in osteoarthritis involves an increase in its expression in both chondrocytes and synovial cells, which affects catabolic and pro-inflammatory genes [57]. Tenocytes respond to mechanical signals by transforming them into biochemical signals via a second messenger such as $Ca^{2+}$ or $IP_3$ [58]. The mechanical load directly regulates gap junction permeability [59]. Some of the Cxs assemble to form hemichannels [60]. Through mechanical stimulation, ATP is released and acts in a paracrine or autocrine manner through the stimulation of $P2Y_2$ purinoceptors, resulting in increased intracellular $Ca^{2+}$ [58]. Cx43 associates with actin to stabilize gap junctions at the plasma membrane [61].

### Cardiac fibroblasts

Cardiac fibroblasts are the most abundant cell type in the heart, play a key role in the myocardial maintenance and repair, and can transform into cardiac myofibroblasts, which are present in valve leaflets in the adult heart. These cells express α-smooth muscle actin (α-SMA) and are referred to as α-SMA-containing stress fibers [62]. The cells are joined by gap junctions that express Cx43 [63], enabling $Ca^{2+}$ signaling that causes the release of $Ca^{2+}$ from the endoplasmic reticulum in response to ATP, histamine, 5-hydroxytryptamine (5-HT) [64] (Lundqvist et al., unpublished), or bradykinin [65]. These cells produce extracellular matrix, exhibit high $Na^+/K^+$-ATPase activity levels in the extracellular matrix [66], and also produce and release a substantial number of cytokines and growth factors into their environment, thereby regulating cell function in an autocrine and paracrine manner [62].

### Hepatocytes

Agonist-evoked $Ca^{2+}$ signals are found in the liver and are manifested as the propagation of intercellular $Ca^{2+}$ waves through liver cells called hepatocytes. Agonist binding to plasma membrane receptors stimulates $G_q$ proteins, which activate PLC and cause $Ca^{2+}$ mobilization from internal stores [67]. The intercellular propagation normally takes place through Cx43-containing gap junctions [68]. ATP

release into the extracellular space stimulates purinoceptors, a paracrine signaling pathway [69].

### Glandular cells

Intercellular signaling in salivary glands has been observed when 5-HT triggers intercellular $Ca^{2+}$ waves through gap junctions and induces $Ca^{2+}$ release via the $IP_3$ receptor [70]. Pancreatic acinar cells in the exocrine part of the gland also conduct intercellular $Ca^{2+}$ signaling between cells [71].

## Inflammation at the cellular level

During inflammation, the expression and affinities of several receptors are changed. In astrocytes, Toll-like receptor 4 (TLR4) expression increases [72, 73], and opioid receptors alter their responses to agonists and antagonists [74, 75].

Furthermore, the cytoskeleton is disrupted into more diffuse and ring-structured actin filaments. Lipopolysaccharide (LPS) exposure alters the actin cytoskeleton in astrocytes [73], macrophages [76], neutrophils [77], and pulmonary monocytes [78]. $Ca^{2+}$ signaling in the astrocyte network is elevated, resulting in increased ATP production and release through the opening of hemichannels. ATP stimulates purinoceptors through autocrine or paracrine mechanisms and results in increased $Ca^{2+}$ release from internal stores; this release occurs in the form of $Ca^{2+}$ oscillations, which may change the balance of $Ca^{2+}$-regulating processes [21]. This extracellular $Ca^{2+}$ signaling attenuates intercellular $Ca^{2+}$ signaling, causing reduced communication via gap junctions [79]. Sodium transporters such as the $Na^+/K^+$-ATPase are downregulated [73]. Neuronal excitability is increased due to the inflammation, leading to increased glutamate release at neural synapses. Astrocytes are the predominant players in clearing glutamate from the extracellular space. The uptake of excessive extracellular glutamate by astrocytes via the membrane-bound glutamate transporters GLAST and GLT-1 plays a critical role in preventing glutamate excitotoxicity [80]. Glutamate uptake transporters are downregulated in the presence of excess glutamate, which leads to the inhibition of glutamate uptake [81]. The increased release of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and IL-1β also occurs [72, 73, 82, 83] (Fig. 3).

Inflammation plays a part in most, if not all, CNS insults. Examples of inflammatory diseases where some of these cellular parameters have been shown to be changed are in the Alzheimer's disease [84], Parkinson's disease [85], multiple sclerosis [86], traumatic injury and

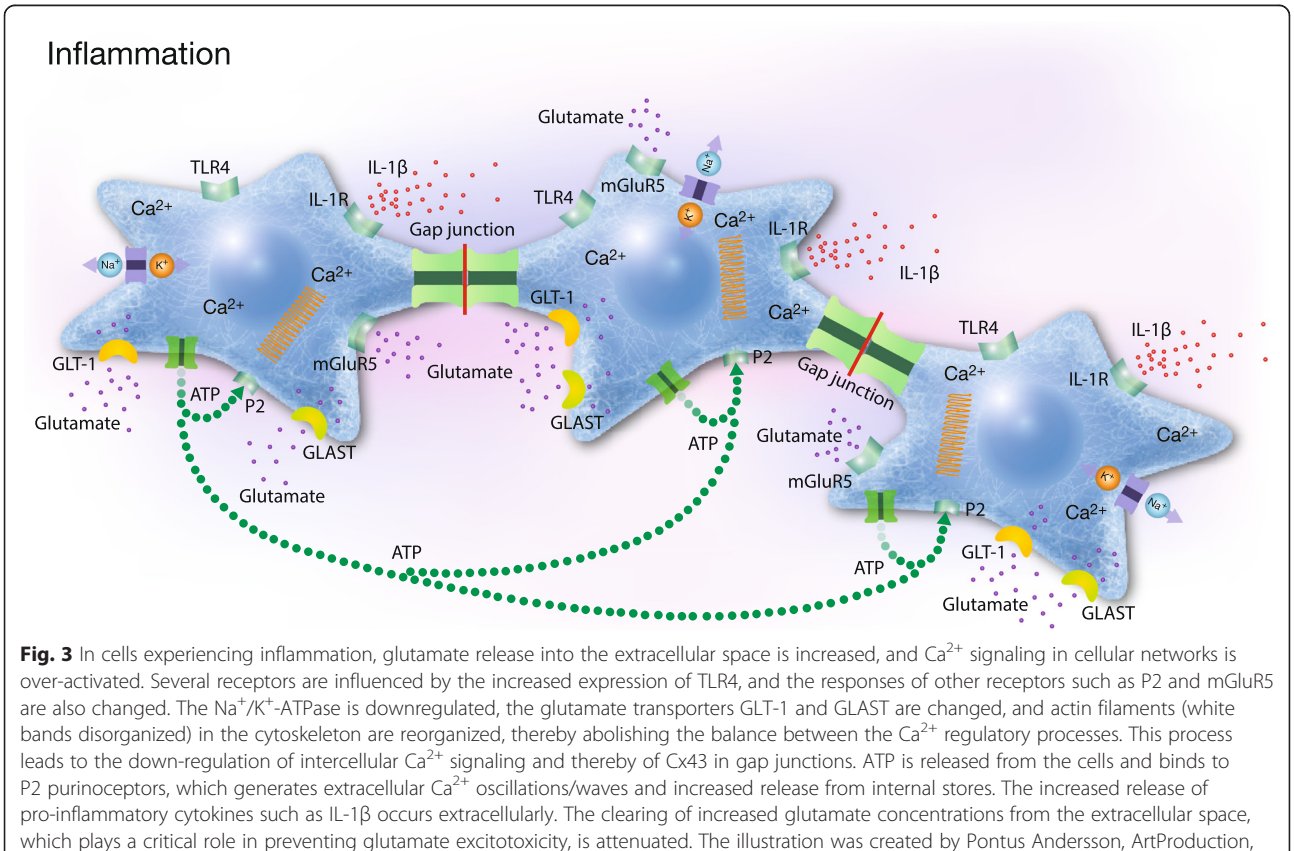


**Fig. 3** In cells experiencing inflammation, glutamate release into the extracellular space is increased, and $Ca^{2+}$ signaling in cellular networks is over-activated. Several receptors are influenced by the increased expression of TLR4, and the responses of other receptors such as P2 and mGluR5 are also changed. The $Na^+/K^+$-ATPase is downregulated, the glutamate transporters GLT-1 and GLAST are changed, and actin filaments (white bands disorganized) in the cytoskeleton are reorganized, thereby abolishing the balance between the $Ca^{2+}$ regulatory processes. This process leads to the down-regulation of intercellular $Ca^{2+}$ signaling and thereby of Cx43 in gap junctions. ATP is released from the cells and binds to P2 purinoceptors, which generates extracellular $Ca^{2+}$ oscillations/waves and increased release from internal stores. The increased release of pro-inflammatory cytokines such as IL-1β occurs extracellularly. The clearing of increased glutamate concentrations from the extracellular space, which plays a critical role in preventing glutamate excitotoxicity, is attenuated. The illustration was created by Pontus Andersson, ArtProduction, Gothenburg, Sweden

ischemia, autoimmune inflammation, and damage and diseases where immune and inflammatory cells have crucial roles in these responses [87].

Changes in cellular parameters due to inflammation are also observed in other cellular networks.

The cellular location of TLR4 in keratinocytes has a key role in the initiation of innate immunity and in the regulation of adaptive immune responses. TLR4 may also be an important regulator of wound inflammation [88] and dermal wound healing [89]. Keratinocytes play a role in the pathogenesis of cutaneous inflammatory disease by producing pro-inflammatory cytokines such as IL-1α, IL-1β, TNF-α, IL-6, IL-8 and granulocyte-macrophage colony-stimulating factor, adhesion factors, and co-stimulatory molecules [90].

In rheumatoid arthritis, the synovial lining cells exhibit an infiltration of macrophages, increased levels of pro-inflammatory cytokines, and upregulation of catabolic matrix-degrading enzymes such as matrix metalloproteinases, which leads to the activation and destruction of cartilage and bone cells. An interaction between LPS, TLR4, and collagen type II in chondrocytes has a role in initiating this pro-inflammatory activity and can lead to the inhibition of cartilage extracellular matrix production and chondrocyte inflammation and apoptosis [91]. The stimulation of chondrocytes with IL-1β causes the significant up-regulation of TLR4 [92], increased production of metalloproteinases, suppression of type II collagen and proteoglycan production and induction of pro-inflammatory mediators such as prostaglandins and nitric oxide [49]. The degradation of articular cartilage is a characteristic feature of arthritic diseases, and IL-1 is one of the more potent cytokines that promotes cartilage catabolism, enhances Cx43 expression and increases $Ca^{2+}$ signaling [47]. Altered Cx43 expression may be an early phenotypic event in osteoarthritis [48]. The concentration of glutamate in synovial fluid is notably increased in both osteoarthritis (a low-grade inflammatoryjoint disease) and rheumatoid arthritis (a chronic autoimmune disease) and is correlated with increased inflammatory mediators such as TNF-α and chemokines [93].

The increased extracellular glutamate concentration is regulated by glutamate transporters in rat chondrocytes [94] as well as in human cartilage [54]. Glutamate may function as an autocrine factor. Ionotropic glutamate receptors contribute to pain and the inflammatory stages of osteoarthritis, and receptor antagonists have been proposed as a potential treatment [95]. Adenosine receptors have important roles in the regulation of inflammation and may be involved in the inhibition of pro-inflammatory cytokine release, especially in chondrocytes and osteoblasts [96].

Cardiac fibroblasts likely have important roles in the inflammatory process in the heart. These cells transform into myofibroblasts in conditions of reduced ventricular function; extracellular matrix production is increased, chemokine production is upregulated, and inflammatory pathways are changed. Furthermore, these fibroblasts recruit monocytes into cardiac tissue [97]. Mechanical stress, which also induces increased chemokine production, appears to be important in the inflammatory process.

During pathophysiological inflammation in salivary gland cells, LPS induces the secretion of IL-β into the saliva. TLR4 is upregulated via the LPS signaling pathway [98]. Inflammation in the mammary gland during lactation leads to TLR4 activation and increased IL-8 secretion [99]. Inflammation in the lacrimal gland results in up-regulation of TLR4 in the corneal epithelium of the eye [100].

TLR4 may be involved in the immune response in liver disease caused by hepatitis B, which can lead to inflammation, cirrhosis, and hepatocellular carcinoma [101].

Airway inflammation is often caused by Gram-negative bacteria or by the presence of endotoxins, which can lead to the development of asthma. TLR4 also seems to play an important role in this process [102].

## Systemic inflammation and the spread of inflammation

Do coupled cell networks in different organs possess a signaling system that can spread or propagate from the coupled cell networks of one organ to those in other organs on either the contralateral or ipsilateral side? Can chronic, low-grade, systemic inflammation influence coupled cell networks in one or several organs, leading to chronic tissue degradation?

Obesity promotes chronic, low-grade, systemic inflammation that contributes to the development of type 2 diabetes, cardiovascular disease and metabolic disorders such as liver steatosis [103, 104]. Both the intestinal microbiota and a high-fat diet have been shown to induce changes in gut homeostasis and consequent mucosal inflammation. Intestinal inflammation may be an early event that leads to the systemic inflammation of several organs [105]. Furthermore, there appear to be strong links between type 2 diabetes, dementia and neurodegenerative diseases such as Alzheimer's disease. Underlying mechanisms involved seem to be defaults in cellular insulin resistance, inflammatory and oxidative stress pathways [106]. Additional autoimmune rheumatic diseases are thought to result in both joint and systemic inflammation, with TNF-α acting as the prominent cytokine. This process may be an initiator of neuroinflammation [5] and might increase the risk of cardiovascular tissue destruction [107]. Transient receptor potential (TRP) channels, expressed in non-excitable coupled cell networks [108, 109] play important roles in physiological as well as in pathological processes; inflammation and pain. They activate signal pathways via $Ca^{2+}$ entry

and membrane depolarization. They contribute to cell volume regulation and are involved in diseases such as osteoarthritis, cardiovascular disorders, type 2 diabetes mellitus etc. [108, 110, 111]. Stimulation of TRP channels can lead to astrocytic reactions followed by activation of nociceptive input [109]. Osteoarthritis is a condition characterized by mainly pain, reduced joint movement and signs of inflammation, such as swelling. TRP channels antagonists have been investigated as a novel therapy by alleviating pain and some inflammatory aspects [112].

Innate immune dysregulation can be the driver for autoinflammatory diseases. It is efforts to define overlapping, maybe genetically determined inflammatory responses in autoimmunity and infection diseases [113]. These questions are important to reflect on, and more studies are required to obtain more conclusive answers.

## Pain processing and mirror pain due to neuroinflammation

CNS glia, especially microglia and astrocytes coupled into networks, appear to be capable of action at a distance. The nervous system can also initiate signals that alter the function of glial cells. Activated glia release immunomodulatory products, which can be key mediators of the modulation of neuronal activity. It may lead to low-grade inflammation at the site of the damaged or affected peripheral nerve(s). Inflammatory receptors are affected, and interactions between these receptors may induce immune signaling changes, which may be of importance in neuroinflammation. Such immune-mediated inflammation can be underlying mechanisms of persistent or long-term pain. Astrocytes and microglia become activated after pain-activating substances are released from neurons located in the spinal cord and brain in response to peripheral and/or CNS trauma, which can lead to over-activation [7] that results in morphological and functional changes. Additionally, sodium transporters down-regulation and cytoskeletal disruption occur, thereby abolishing $Ca^{2+}$ signaling by altering the balance between $Ca^{2+}$-regulating processes [114].

Changes in the release of pro- and anti-inflammatory cytokines are observed in many clinical studies [83, 115]. These mediators can underlie the spread of the neuroinflammation and pain to the uninjured side [8]. Effects on contralateral non-lesioned structures have been well-documented, and these effects also occur on the ipsilateral side. Unidentified signaling mechanisms linking either the same side of the body or the two sides of the body likely exist [18]. Glial cells and potentially astrocytes can participate in the activation and initiation of signals that regulate neuronal function but may also initiate signals in other parts of the body.

## Conclusion

Coupled cell networks throughout the body are coupled by gap junctions expressing Cx43 and $Ca^{2+}$ signaling systems. These cells are targets in conditions that can lead to immune-mediated inflammation. Several basal cellular parameters are then changed. One hypothesis is that coupled cell networks use a signaling mechanism between the cells within their own networks. The signaling can be transferred to other coupled cell networks in other organs. This form of communication may give rise to inflammatory systemic diseases. Such signaling may facilitate the spreading of pain to sites both contralateral and ipsilateral to the original injury site; this spreading is sometimes observed with long-term pain.

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

EH and ES participated in the design of the study and wrote this review together. Both authors read and approved the final manuscript.

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#### Highlights

Inflammatory processes result in biochemical changes and altered functions in coupled cell networks in different organs in the body.
Coupled cell networks express gap junctions comprising connexin 43 and inter- and/or extracellular $Ca^{2+}$ signaling systems.
Signaling between coupled cell networks can facilitate the spread of inflammation, which can lead to pain and pain spreading on both the ipsilateral and contralateral sides if nervous tissue cells are involved.
Chronic, low-grade systemic inflammation can influence coupled cell networks in one or several organs, leading to chronic tissue degradation.

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