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The role of nociceptive synaptic transmission modulation
Modulace nociceptivního synaptického přenosu na míšní úrovni

Bachelor´s thesis

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Podpis

Abstract

Chronic pain phenomenon is an important problem in modern medicine. Occurring of this phenomenon is tightly connected with nociceptive transmission and modulation of nociceptive signal on the spinal cord level. Under the pathological conditions such as injury or inflammation this modulation is affected by different types of endogenous molecules with pain enhancing attributes. Important group of these molecules are chemokines, immune system substances, also responsible for immune cells recruitments. However, in pathological states chemokines show ability to modulate nociceptive signal and induce chronic pain. CCL2, in particular, has a significant role in modulation of these processes in the spinal cord. Investigation of the mechanisms by which CCL2 influences the spinal cord and dorsal root ganglion may be an important part for preventing the development of chronic pain.

Key words: nociception, pain, spinal cord, chemokines, CCL2

Abstrakt

Fenomén chronické bolesti je významným problémem současné medicíny. Vznik tohoto fenoménu těsně spojen s nociceptivním přenosem a modulací nociceptivního signálu na míšní úrovni. Za patologických podmínek jako je úraz nebo zánět je tato modulace ovlivněna působením různých endogenních molekul s bolest zesilujícími účinky. Důležitou skupinou mezi těmito molekulami jsou chemokiny – látky produkované imunitním systémem, které jsou mimo jiné zodpovědné za atrakci imunitních buněk. Nicméně, u patologických stavů mají chemokiny schopnost modulace nociceptivního signálu a indukce chronické bolesti. CCL2, zejména, má významnou roli v modulačních procesech na míšní úrovni. Zkoumaní mechanismů působení CCL2 na úrovní míchy a ganglii může být jedním s klíčů pro prevenci vývoje chronické bolesti.

Klíčová slova: nocicepce, bolest, mícha, chemokiny, CCL2

Content

List of abbreviations

4-HNE	4-hydroxy-2-nonenal
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ASIC	acid-sensing ion channel
ATP	adenosine triphosphate
Ca^{2+}	calcium ion
CCL16	C-C motif chemokine ligand 16
CCL17	C-C motif chemokine ligand 17
CCL2	C-C motif chemokine ligand 2
CCL22	C-C motif chemokine ligand 22
CCL3	C-C motif chemokine ligand 3
CCL5	C-C motif chemokine ligand 5
CCL8	C-C motif chemokine ligand 8
CCR2	C-C chemokine receptor type 2
CCR4	C-C chemokine receptor type 4
CGRP	calcitonin gene-related peptide
Cl^-	chloride ion
CNS	central nervous system
Cox-2	cyclooxygenase-2
CXCL1	C-X-C motif ligand 1
DEG/ENaC	degenerin/epithelial Na^+ channel
DRG	dorsal root ganglion
GABA	gamma-aminobutyric acid
GFAP	glial fibrillary acidic protein
IB4	isolectin B4
IL-1	inteleukin-1
IL-1β	interleukin-1- beta

IL-6	interleukin-6
INFγ	interferon gamma
JNK	c-jun-N-terminal kinase
K^+	potassium ion
KCl	potassium chloride
KCNK	potassium channel subfamily K
LPS	lipopolysaccharide
MCP-1	monocyte chemoattractant protein 1
Na^+	sodium ion
NGF	nerve growth factor
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NS	nervous system
NSCC	nonselective cationic conductance
PKC	protein kinase C
PLC	phospholipase C
PTX	pertussis toxin
RET	receptor tyrosine kinase
ROS	reactive oxygen species
SP	substance P
TNFα	tumor necrosis factor alfa
TRK	tyrosine kinase
TRPA1	transient receptor potential ankyrin 1
TRPM8	transient receptor potential melastatin 8
TRPV1	transient receptor potential vanilloid 1
TRPV2	transient receptor potential vanilloid 2

1. Introduction

This thesis is a theoretical overview of the basis of pain signaling pathways and some important mechanisms involved in the nociception process. According to the International Association for Study of Pain (IASP), pain is defined as a "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". It is always a subjective feeling, which is tightly connected to person's physiological and psychological condition.

We will stay focused on the chronic pain phenomenon, as it no longer has its evolutionary function of warning us from danger, but becomes a problem itself. Acute pain has important functions in our body and organism's homeostasis: being one of the first physiological reactions to a critical stimulus it signalizes body damage and may save us from harm that follows. It only lasts for hours, maximum days. We call the pain chronical when it lasts more than 3 months (according to some sources more than 6 months). Chronic pain has lost its warning functions and no longer helps but becomes a physical and mental burden for many people all over the world. Importance of pain study is thus not only in scientific interest but also in finding a new way to treat people suffering from it.

Chronic pain can be *nociceptive* (caused by inflammation or tissue damage and following activation of special receptors for the pain stimuli – nociceptors), *neuropathic* (caused by a direct damage of the peripheral or central nervous system) and *"other"* (caused by the other factors (e.g. fibromyalgia) ('The Basic Types of Pain'). Sometimes different kinds of pain can occur together and create even more problems for diagnosis and cure. This thesis will be mostly focused on the role of nociceptive signal modulation in development of these pain states and on the role of chemokine CCL2 in this process.

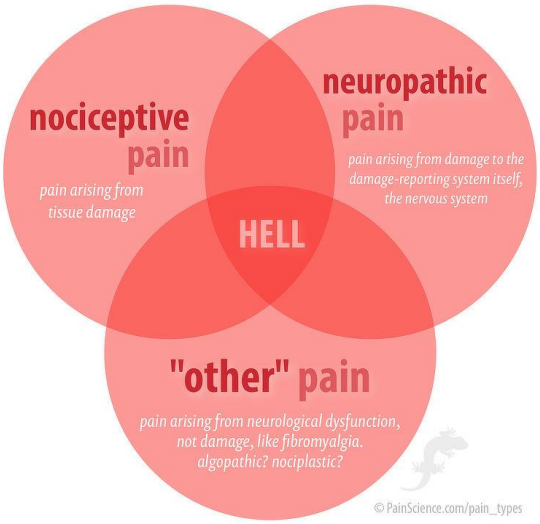

Fig. 1 Types of chronic pain (adapted from 'The Basic Types of Pain')

2 Nociception

As it was mentioned before, pain is a subjective feeling and pain perception is a complex phenomenon. Processing of the pain signal in CNS can vary from person to person. Nevertheless, physiological part of pain perception proceeds according to the certain mechanisms, which are the same for most of the humans. Complex of these mechanisms is called nociception. Nociception is a neurophysiological term, which describes specific actions used for the pain signal transmission. Nociception is a complex process, which includes nascence of the impulse by nociceptors excitation, its transfer by the primary afferent fibers to the spinal cord and then through the spinothalamic pathway to the brain, and its further processing in different cortical centers including somatosensory cortex. Therefore, the nociception process is basically separated into four main parts: signal transduction, transmission, spinal modulation and perception. ('Miller-Keane Encyclopedia & Dictionary of Medicine, Nursing & Allied Health - 7th Edition')

2.1 Detection of a noxious stimuli by nociceptors

Nociceptors are specialized sensory receptors on the peripheral endings of the primary afferent fibers responsible for detection of a pain signal. Nociceptors mediate perception of noxious (unpleasant) stimuli and its transduction into changes of membrane potential, which induces nascence of the action potential after reaching a certain threshold.

Regarding the location of nociceptors, we can divide them into external (laying under the skin, in cornea or on inner organ‘s membranes) and internal (laying in different organs and tissues like muscles, joints, guts etc.)

By the activating (noxious) stimuli, nociceptors can be divided into

- *Thermal* (activated by temperature > ~40°C–45°C or < ~15°C)
- *Mechanical* (activated by pressure)
- *Chemical* (activated by chemical stimuli),
- *Silent* nociceptors (activated only in case of real tissue damage by inflammatory mediators)
- *Polymodal* nociceptors, which are able to recognize a several types of stimuli.

Detection of the signal takes place in receptors (ion channels) located on peripheral nerve endings.

Nociceptor’s capability to react to stimuli depends on the kind of ion channels (receptors) expressed in the cell membrane. Most of the channels are able to react only to a few stimuli and also one cell can express several kinds of receptors.

The most common receptors related to the pain transduction are:

- *Transient receptor potential vanilloid 1(TRPV1)* receptors can be activated by heat (>43 C), chemical stimuli (capsaicin) and response to the wide range of proalgesic and proinflammatory agents.
- *Transient receptor potential melastatin 8 (TRPM8)* receptors are sensitive to menthol and cold (<30-15°C activates Aδ and C afferent fibers)
- *Acid-sensing ion channels (ASIC)* receptors activated by acidic milieu (pH 5.0-6.9)
- *Transient receptor potential ankyrin 1* (TRPA1) responds to cold and chemical irritants (like formalin, menthol, eicosanoids).
- *P2X receptors* are sensitive to extracellular ATP (Chizh and Illes 2001)

A few kinds of other receptors are also candidates for responding to the noxious mechanical stimuli: DEG/ENaC, TRPV2, KCNK. Nevertheless, during the pathological states like hyperalgesia, other receptors can show nociceptive reaction to mechanical stimuli (TRPA1) (Basbaum et al. 2009)

Achieving of the certain threshold by the stimuli leads to the opening of cation channels on the cell membrane of free nerves endings and positively charged ions (Ca^{2+}, Na^{+}) flow inside causing membrane's depolarization and creation of the action potential. This process can be also triggered by the straight mechanical damage of the neuron fiber (Julian and Goldman 1962).

Higher transduction of nociceptive stimuli can be caused by inflammatory mediators like bradykinin, cytokines and others, which are increasing the sensitivity of the nociception related receptors on the nerve ends. Latter mechanism of transduction plays an important role also in the development of the chronic pain. (Pethő and Reeh 2012)

2.2 Signal transfer by primary afferent fibers

Afferent fibers are responsible for signal transferring from peripheral nerve endings to the higher levels of the nervous system (spinal cord via dorsal roots). There are two types of nerve fibers in humans and mammals that mediate pain signal transfer under normal conditions:

Aδ-fibers – thinly myelinated fibers, with conduction velocity about 2-30 m/s, are mostly responsible for sharp pain feeling.

C-fibers – unmyelinated fibers, with conduction velocity < 2 m/s, are mostly responsible for dull pain feeling.

C-fibers are mostly polymodal, they have sensitivity for both heat and mechanical stimuli. Nevertheless, there is also a group of mechanically insensitive, heat-responsive C-fibers (silent nociceptors), which are getting mechanical sensitivity only in case of injury (Recognition and Alleviation of Pain in Laboratory Animals 2009).They are more responsive to chemical stimuli like capsaicin or histamine.

Peripheral afferent fiber's cell bodies are assembled in structures known as dorsal root ganglions (DRG) where they are separated from each other by satellite glial cells (SGC). DRG cells play an important role in signal modulation and are involved in the process of

development of the chronic pain. DRG neurons are also called pseudounipolar. Their axon splits into two branches, one brings the signal from the peripheral nociceptors to the cell body, and the second one connects the cell body with the spinal cord. Those axons are connected via T-shaped junction with the cell body. This structure provides distributing of proteins synthetized in the cell body to both peripheral and central directions. Both branches are also sensitive to different endogenous molecules (lipids, neurotransmitters etc.). Unmyelinated axons of C-fibers tend to make a structure known as Remak bundles, where the Schwann cell surrounds the axons and bundles them, preventing their contact. Those bundles can contain a few different types of C-fibers and single fibers can have an effect on each other in case of nerve damage or other pathology.

In rodents, we can distinguish two major types of C fibers: peptidergic C fibres expressing the peptides calcitonin gene-related peptide (CGRP) and substance P and non-peptidergic C fibers. Peptidergic C fibres depend for their integrity on nerve growth factor (NGF) and express the NGF receptor tyrosine kinase (TRK). They project to the superficial dorsal horn at laminas I and II and terminate on projection neurons of the spinothalamic tract. In non-peptidergic C fibres TRK is downregulated, but another receptor tyrosine kinase (RET) is upregulated. These neurons express Ret receptor, which binds the lectin IB4, and project to the central part of the dorsal horn at lamina II, where they terminate on interneurons. (Zylka, Rice, and Anderson 2005; Braz et al. 2005; Gold and Gebhart 2010)

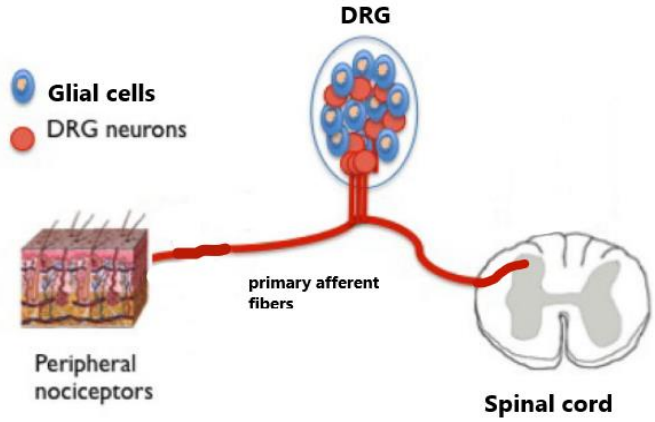

Fig. 2. Afferent fiber's and DRG localization in the mammalian body (adapted from Krames 2015)

2.3 Transmission of the signal to the CNS

2.3.1 Spinal cord

Spinal cord is an important part of central nervous system. Its structure reminds a butterfly's wings, with the white matter on the outside made of axons of descending and ascending tracts and grey matter made of neuronal and glial cell bodies in the inner area.

Grey matter.

Grey matter of the spinal cord is made of neuronal and glial cells bodies. This area has been subdivided in 10 laminae by Rexed. I- IV laminae are situated in the dorsal horn; V-X laminae are placed at the base of the dorsal horn and the central region of the ventral horn. Lamina I is made of thin layer of marginal cells located at the border with small local circuit neurons of substantia gelatinosa, which form II and III laminae. Lamina IV consists of larger cells of the nucleus proprius and lies in the center of the dorsal horn. Nucleus proprius cells are also presented in laminae III and V. This structure contains the first synapse of the spinothalamic tract carrying pain and temperature signal. Lamina V has mainly neurons involved in processing sensory afferent stimuli from Aδ nociceptors (skin, muscle, joints and visceral nociceptors). This area also contains interneurons and propriospinal neurons (their axons terminate within spinal cord). Lamina VI neurons are mostly responsible for "fast pain" and flexion reflex. In lamina VII can be found important structures participating in proprioception. Lamina VIII consists of a group of commissural neurons and their axons are sent to the ventral white commissure. Lamina IX is formed by the groups of large neurons laying in the ventral horn. Small, tightly packed neurons around the central canal are forming lamina X (grey commissure). Laminas I and II are the most interesting for us, as that's the place where C fibers project. Laminas V, VI and X are also participating in nociceptive processes. (Rexed 1954)

Spinal projection of nociceptive signal is regulated by different mechanisms like local inhibitory and excitatory interneurons (e.g. GABA-immunoreactive interneurons, providing pre- and post-synaptic inhibition), NMDA receptors activation and descending impact from the brain stem (excitatory and inhibitory). But during pathological states this regulation is interfered, usually causing increased sensitivity of the incoming afferent fibers and occurring of central sensitization.

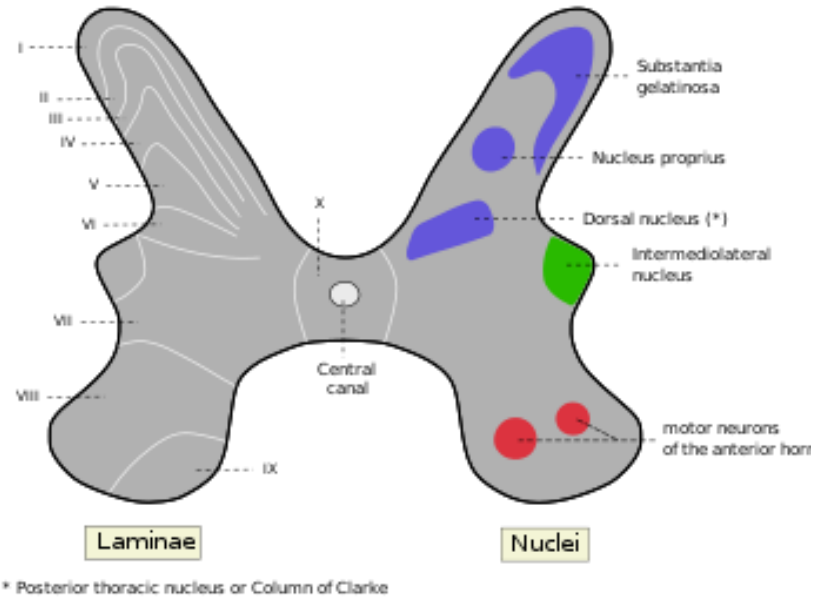

Fig. 3. Spinal cord's structure with laminae by Rexed (left side) and significant nuclei (right side) (adapted from Wikipedia, 2006)

White matter

As mentioned before, white matter consists of axons. White matter of the spinal cord consists of projection tracts – pathways, which carry the signal from the spinal cord to the cerebral cortex (ascending sensory tracts) or in the opposite direction (descending tracts). It can be divided into parts (columns). Posterior column together with medial lemniscus form the pathway, which mostly consists of the ascending projections of the pseudounipolar neurons of the DRG and carry the sensory information (e.g. proprioception vibration). Anterior (ventral) white commissure is situated anterior to the grey commissure (Rexed lamina X) and contains project neurons of the spinothalamic tracts, which cross sides there and transfer pain, temperature and pressure signals to the brain. Anterolateral column consists of neurons of spinocereberall tract, by which conveyed proprioceptive, pressure and touch signals. Descending corticospinal (pyramidal) tract is divided in white matter into anterior corticospinal tract, where it neurons pass through the anterior white commissure and terminate on motor neurons in the grey matter and lateral corticospinal tract located between the posterior grey column and the spinothalamic tract. (Grey's Anatomy, 41st Edition)

After being conveyed to projection neurons in the spinal cord, noxious signal is led to higher structures of brain stem and thalamus by ascending tracts.

2.3.2 Ascending and descending tracts

There are two main nociceptive *ascending pathways* arising from the spinal cord distinguished: the spinocerebellar tracts and the anterolateral system. Spinothalamic tract is the part of the anterolateral system and arises from lamina I and IV-IX. Projection neurons of the spinothalamic tract are terminating on the neurons in the thalamus, where the signal is then transmitted further into the somatosensory cortex. Some of them are branching into the spinoreticular tract, terminating in the brain stem and then carrying the signal through the amygdala to the cingulate and insular cortex. Noxious information brought to the somatosensory cortex is elaborated for sensory-discriminative aspects of the pain like localization and intensity, when cingulate and insular cortex processing is associated with emotional part of the pain experience. (Apkarian et al. 2005)

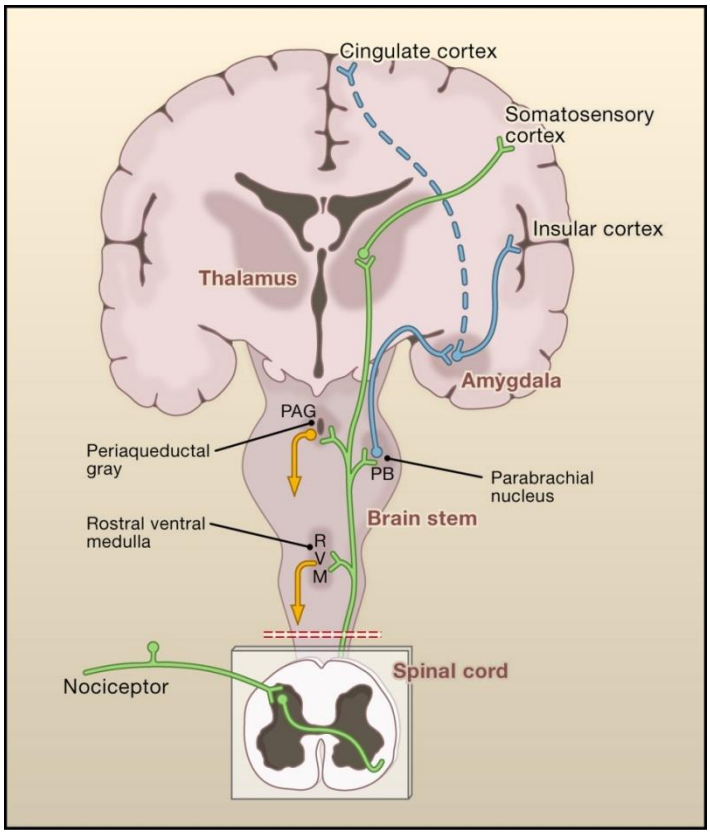

Fig. 4. Ascending pathways schema. Spinothalamic tract (green) with branches to the spinoreticular tract (blue) (adapted from Apkarian et al. 2005)

Descending pathways are bringing signal from the brain to the spinal cord, regulating processes in it. Nociceptive descending regulation is mostly provided by a complex known as rostral ventromedial medulla located in the brainstem. Serotoninergic and noradrenergic neurons from this complex project to the dorsal horn of the spinal cord and modulate nociceptive signal directly affecting primary nociceptive fibers or indirectly through the interneurons. They project to the superficial part of the dorsal horn and have inhibitory effect on C-fibers nociceptors and no inhibitory or even enhancing impact on Aδ-fibers activity. ON-cells and OFF-cells are distinguished in the descending nociceptive modulation and their activation leads respectively to excitatory and inhibitory effects on the spinal cord level. They are receiving the signal from the midbrain periaqueductal gray

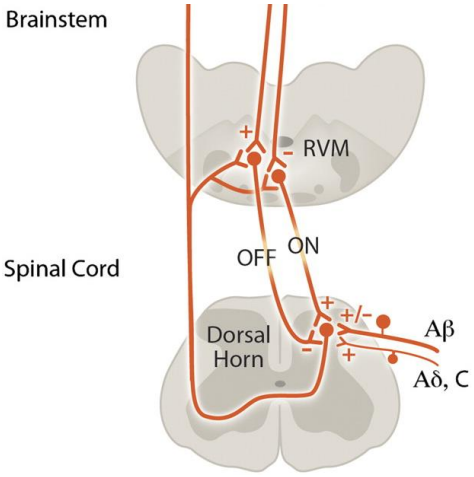

Fig. 5. Descending pathways with inhibitory (OFF) and excitatory (ON) efferent neurons. (adapted from Mendell 2011)

structure. ON-cells during the injury or inflammation are activated by pain-enhancers (e.g. prostaglandins) and cause hyperalgesia, when OFF-cells have analgesic effect and are activated by pain-silencing molecules (e.g. opioids). There is a negative regulation in the activity of these cells. (Heinricher et al. 2009; Fields, Malick, and Burstein 1995)

3 Glia

Glial cells are non-neuronal cells presented in the nervous system and tightly connected with its functioning. Their functions include providing protection of the nervous tissue by forming the myelin layer and cleaning debris, delivering nutrients and oxygen to the neurons, maintain homeostasis and forming the main part of NS immune system. They also participate in the nociceptive processes in different ways for example by synthetizing and releasing glial mediators (cytokines, chemokines growth factors and proteases) (Ji, Berta, and Nedergaard 2013). In case of injury or inflammation glial cells contribute to the pain sensation by releasing endogenous substances as NO, ATP, prostaglandins, excitatory amino acids, interleukins and tumor necrosis factor, which are act as pain enhancers.

Glial cells are divided by their localization into two groups: CNS glia and PNS glia.

3.1 Central nervous system glial cells

1. Astrocytes are star-shaped cells supplying nnutrients and oxygen to neurons and participating in the synapse processes and its modulation, releasing transmitters like glutamate, ATP, pro-inflammatory cytokines (e.g. interleukin-1 beta, tumor necrosis factor alpha), chemokines (CCL2, CXCL1) and growth factors (brain-derived neurotrophic factor, basic fibroblast growth factor). Those cells are able to connect and communicate through the gap-junctions, which provide fast reactions to the environment changes. Astrocytes can express glial fibrillary acidic protein (GFAP), which is mostly found in reactive astrocytes

responding to CNS injures. GFAP then serves as the main marker for detecting activated astrocytes.(Sofroniew and Vinters 2010)

2. Microglial cells are capable of phagocytosis and are distributed throughout the CNS. They play an important role in the brain immune system, as they are able to release and to respond to numerous cytokines and chemokines. Activated microglia can release different pro-inflammation factors such as TNFα, IL-1 and IL-6. In case of injury of peripheral afferent fibers, ATP is released and binds to microglia's purinergic receptors activating the cells. Microglia can be also activated indirectly by astrocytes and through their products. Glial cells activation leads to the enhancement of neuronal central sensitization and nerve injury-induced persistent pain. (Tsuda et al. 2003)

3. *Oligodendrocytes* are smaller cells with higher density, which form myelin sheath in the CNS. Latter is providing electrical insulation to the axons. Oligodendrocytes can form a myelin sheath for a several cells and one neuron can be myelinated by several oligodendrocytes making a net structure in CNS. (Baumann and Pham-Dinh 2001)

4. *Ependymal cells* or ependymocytes are involved in production of the cerebrospinal fluid. They line ventricles with the fluid in brain and the central canal of the spinal cord.

3.2 Peripheral nervous system glial cells

1. *Schwann cells* are similar to the oligodendrocytes in the CNS in functions, as they also provide myelination of axons, but unlike the oligodendrocytes, Schwann cells myelinate only one axon. Schwann cells also play a role in the immune system, as they are capable of phagocytosis (Reichert, Saada, and Rotshenker 1994) and antigen representation (Horste et al. 2010).

2. *Satellite glial cells* are similar to the astrocytes in the CNS as they are also providing nutrients to the neurons. These cells are presented in the dorsal root ganglions, have gap-junction connections, respond to ATP, are sensitive to injuries and inflammation and are involved in occurring of pathological states, such as chronic pain. (Hanani 2005)

4 Neurotransmitters and modulators

Neurotransmitters are chemicals, which are presented at the synapses and implement the signal passing between neurons. They are released at the end of one neuron and bind on the receptors of the other neuron (target neuron).

Neurotransmitters can be divided by the affection type to excitatory and inhibitory. By the chemical structure we can distinguish several types: amino acids, gasotransmitters, monoamines and acetylcholine, peptides, purines and others (Hyman 2005). In this thesis, we will stay focused on those, which are participating in the pain signal transmission.

4.1 Amino acids

Glutamate is the main excitatory neurotransmitter in the CNS. It is released on synapses and binds to several kinds of receptors on the target neuron membrane. They are either ionotropic or metabotropic. Activation of ionotropic leads to increased permeability of ion channels for Na^+ and K^+ or for Ca^{2+} and membrane depolarization on the target neuron. There are three types of ionotropic receptors that glutamate can affect:

- NMDA receptors activated by binding both glutamate and glycine and causing depolarization by increasing permeability for Ca^{2+} and Na^+ ions.
- Kainate receptors with excitatory postsynaptic and inhibitory presynaptic activity
- AMPA receptors providing fast synaptic transmission in the CNS.

Metabotropic type of glutamate receptors are G-protein coupled receptors, which activation induces second messenger system in the cell. (Purves et al. 2001b)

GABA (gamma-aminobutyric acid) is the main inhibitory neurotransmitter in the CNS, but in some cases, it can depolarize membrane and have an excitatory effect (Wang et al. 2015). Its binding to the neuron receptors increase permeability for Cl^- ions flowing inside the cell and K^+ ions flowing out and causes hyperpolarization. There are two types of receptors for GABA: $GABA_A$ receptors are the part of the ligand-gated ion channel complex and $GABA_B$, which are metabotropic, G-protein coupled receptors modulating ion-channels function through intermediates. $GABA_A$ receptors on primary afferent fibers provide pre-synaptic inhibition in the spinal cord, while located on the spinal cord neurons they ensure post-synaptic inhibition. (Olsen and DeLorey 1999)

Glycine is an inhibitory neurotransmitter in CNS (especially in the spinal cord, brainstem and retina), inducing hyperpolarization by opening Cl^- ion channels, and has an analgesic effect in nociceptive processes (Cheng et al. 2009). However, it can play a role in excitatory mechanisms, as it's a co-agonist along with glutamate for NMDA-receptors. (Mayer, Jr, and Clements 1989)

4.2 Gasotransmitters

NO (nitric oxide) is a messenger molecule, which modulates the release of other neurotransmitters. Its synthesis can be activated in phagocytes, as a part of immune response. In the spinal cord NO may reduce nociceptive transmission. (Bavencoffe, Chen, and Pan 2014)

4.3 Monoamines and acetylcholine

Dopamine is an organic chemical involved in various neuronal processes in the CNS and is one of the main neuromodulators in the brain. In the periphery it has a function of a local messenger in different tissues, including decreasing of activity of lymphocytes in the immune system. (Ch Beck et al. 2004)

Serotonin is a neurotransmitter, found in gastrointestinal tract, blood and CNS. Serotonin receptors on the peripheral and central nervous system mediate both excitatory and inhibitory neurotransmission and modulate release of other neurotransmitters.

Acetylcholine is one of the main excitatory neurotransmitters in the body and also a neuromodulator in brain. It has several types of receptors in the human body: muscarinic acetylcholine receptors are found in both central nervous system and in peripheral nervous system of the heart, lungs and gastrointestinal tract; nicotinic acetylcholine receptors muscle-type which are located at the neuromuscular junctions; nicotinic acetylcholine receptors neuronal-type which are expressed in autonomic ganglia of the central and peripheral nervous systems.(Purves et al. 2001a) In brain acetylcholine is involved mainly in processes of memory, attention and motivation.

4.4 Peptides

Substance P (SP) is mainly released from neurons and is involved in important physiological processes including inflammation and nociception. It provides interactions between neurons and immune system cells by endocrine or paracrine signaling, influencing proliferation rates in immune cells and cytokine production. (McGillis, Organist, and Payan 1987)

Calcitonin gene-related peptide (CGRP) function depends on the cell type of its release. When released in the ventral horn of the spinal cord from motoneuron cell bodies it acts as a vasodilator and contributes to the nerve regeneration after the injury. When released from the dorsal root ganglion cells, CGRP induces nociceptive transmission (Chen et al. 2010).

CGRP also contributes to the sensitization of peripheral afferents in pathological states. (Walsh, Mapp, and Kelly 2015)

4.5 Purines

ATP has a wide range of functions in the human body including neurotransmission. ATP may be released from different cells as a physiological or pathophysiological response to mechanical stress, hypoxia, inflammation and some agonists. It is involved in many stages of pain signal transfer. ATP can bind to its receptors predominantly found on non-peptidergic afferent neurons of dorsal root ganglion and also act as a neurotransmitter in the central pain pathways, released from the terminals of the primary afferent fibers at the synapses in the spinal cord. (Burnstock 2006)

4.6 Cytokines

Some of neurotransmitters described below can also function as pro-inflammatory factors, especially released during pathological states. Nevertheless there are other various endogenous molecules that are having pro-inflammatory capabilities and are highly involved in nociceptive processes. The most prevalent group of these molecules is formed of cytokines.

Cytokines are a group of cell signaling molecules taking part in immunomodulation and also, as we will see, in neuromodulation. This group includes different kinds of chemokines, interferons, interleukins, tumor necrosis factors etc. In this thesis we will talk about those related to the nociceptive transmission.

Significant role in pain modulation plays *tumor necrosis factor alfa (TNFα)* – pro-inflammatory agent, released from macrophages and other immune cells after stimulation by lipopolysaccharides (LPS), viruses, mitogens, parasites and other cytokines. It's binding to the cell receptors triggering cell pathways that activate transcription factors necessary for cell proliferation and differentiation, secretion of other cytokines and inflammatory response. TNFα also modulates sensitivity of neuron receptors and has impact on glial cells activity. (Leung and Cahill 2010)

Another substantial cytokine is *interleukin-1 beta (IL-1β)* also secreted by activated macrophages and other immune cells and acts as a hyperalgesic agent activating astrocytes and microglia and participating in prostaglandins release. (Sung et al. 2012)

IL-1β belong to the important subgroup among cytokines, to which the rest of this thesis will be dedicated - chemokines.

5 Chemokines

Chemokines are cytokines capable of chemotaxis that determine immune cells placing and migration. Small peptide molecules (~7-15 kDA), they bind to two types of receptors on the membrane of the target cell: G-protein coupled chemokine receptors, and atypical chemokine receptors which are forming chemokine gradient and temper inflammation. (Griffith, Sokol, and Luster 2014)

Some of them are constantly presented in the body providing homeostatic immune cells migration, when the others are only released under pathological conditions and attract monocytes and other immune cells to the site of inflammation. The latter are released in response on pro-inflammatory factors like TNFα, IFNγ, IL-1 or LPS(Schwarz et al. 1997; Wu et al. 2011). Depending on the location and occasion of secretion chemokines may be involved in different physiological processes and show different functions from contributing to peripheral nerves regeneration to developing of pathological states like hyperalgesia (Kwon et al. 2015; Pflücke et al. 2013). Chemokines are involved in many biological pathways in the cell and their activity mostly leads to leukocyte migration or release of other cytokines.

By the structure we can divide chemokines to several classes according to the spacing and number of cysteine residues in N-terminals of protein: CC class (or beta-chemokines) with two adjacent cysteines, CXC class (alfa-chemokines) with cysteines separated by one amino acid, CX3C (delta – chemokines) with 3 separating amino acids and C with just two cysteines in its structure (gamma – chemokines). (Rollins 1997)

In nervous system chemokines may be produced and released by glial cells and neurons. These cell types also express chemokine receptors and are able to modulate neuronal activity. Therefore, chemokines are not only participating in immune processes but also involved in neuronal system modulation activities. Being a pro-inflammatory factor they shown to increase sensitivity of nociceptors. (Pflücke et al. 2013)

6 Chemokine (C-C motif) ligand 2 (CCL2) (former name Monocyte chemoattractant protein 1(MCP1))

Latest studies show a significant impact of CCL2 chemokine on pathological pain processes. In this thesis we will take a closer look on this chemoattractant and its functions in nociception on the spinal cord level.

6.1 Structure

CCL2 belongs to the CC chemokines class so it has four cysteines in its structure which are forming disulfide interactions. Molecular structure of the chemokine is made of three distinct domains: a very flexible N-terminal domain, constrained by disulfide bonding between the N-terminal cysteines; a long loop that leads into three antiparallel β-pleated sheets; and an α-helix that overlies the sheets. The main chain conformation of two first cysteines also has an impact on the quaternary structure of the protein. The final protein structure is a dimer.

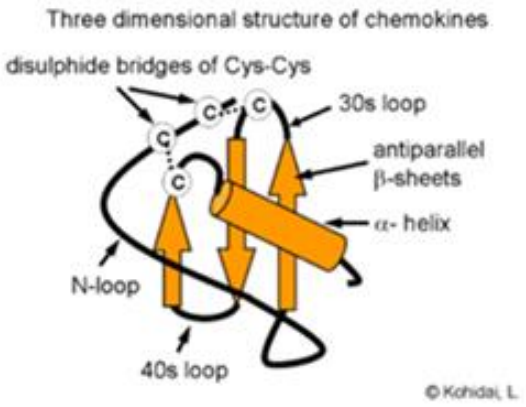

Fig. 6. Structure of the CCL2 monomer (adapted from Kohidai, L 2006)

6.2 Receptors

CCL2 binds to two kinds of receptors: CCR2 and CCR4 receptors (Monteclaro and Charo 1997). They are G-coupled proteins which are expressed on the surface of cells attracted by CCL2 (e.g. $CCR2^{+}$ macrophages, T-cells etc.) and have an ability to bind other structurally similar chemokines (e.g. CCL3, CCL5, CCL8, CCL16, CCL17 and CCL22). However, CCR2 has the highest affinity for CCL2 ligand and is the main receptor for this chemokine. There are two types of CCR2 receptors: CCR2A (10%) and CCR2B (90%) which only differ in their terminal carboxyl tails. They are transcribed from the same gene, but alternatively spliced. Depending on the receptor type cellular responses on CCL2 may differ (Wong et al. 1997). During the injury expression of the CCR2 is up-regulated in response to increased CCL2 levels both in nociceptive and non-nociceptive fibers and leads to hypersensitivity of the latter, contributing to the pain maintenance. (White et al. 2005)

6.3 Antagonists

One of the ways to determine the influence of CCL2 on neuronal processes used in its studies is applying an antagonist of CCR2 receptors and confirmation of its inhibiting effect. Mostly antagonists used for this purposes are synthetically developed (e.g. INCB3344, RS504393) (Dansereau et al. 2008; Baamonde, Hidalgo, and Menéndez 2011; Zhu et al. 2014)

6.4 Secretion

CCL2 is present in cells of different parts of the nervous system constitutively and may modulate neuronal activity and neuroendocrine functions (Rostène, Kitabgi, and Parsadaniantz 2007).

However, during the pathological states it can be released by neurons and glial cells in response to various stimuli: other pro-inflammatory factors like TNFα, capsaicin, potassium, ATP, SP and CGRP. CCL2 secretion, localization and final effect vary in different pain models. For instance, type of the injury may define whether or not CCL2 that is synthetized in DRG neurons will be released from the afferent fibers in the spinal cord (Jeon, Lee, and Cho 2009). Secretion of CCL2 in calcium-dependent manner from the DRG neurons in the dorsal horn was observed after KCl or capsaicin stimulation. There it's co-localized with vanilloid TRPV1 receptors and DRG axon terminals positive for pain-related substance P and CGRP peptides. Distribution of CCL2 in the spinal cord is restrained mostly within the II lamina by Rexed, the place of terminating of the nociceptive afferent fibers. Thus, co-localized with synapses, CCL2 has capability to modulate the nociceptive signals in the spinal cord. (Dansereau et al. 2008)

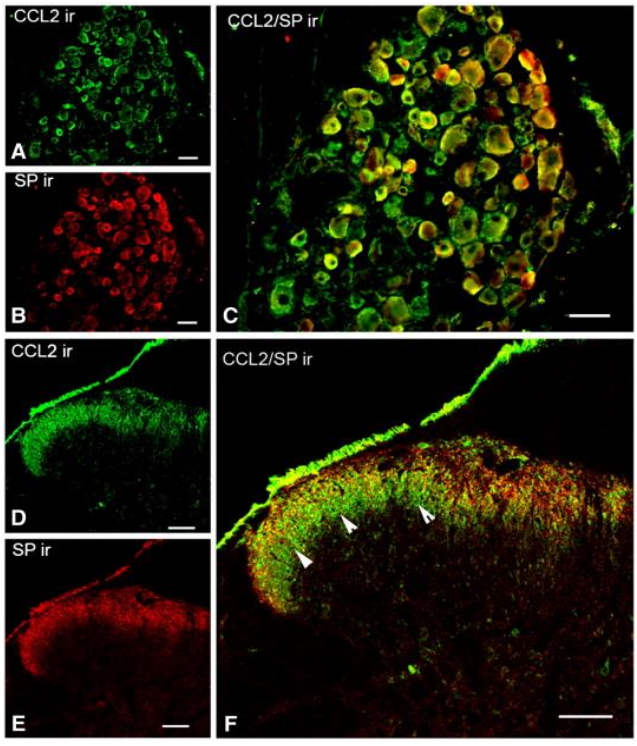

Fig. 7. Immunohistochemical localization of CCL2 (green) and substance P (red) in the DRG (A, B, C) and in the laminae II of dorsal horn of the spinal cord (D, E, F). Co-localization shown in yellow (C, F) (adapted from (Dansereau et al. 2008))

Astrocytes in the spinal cord are capable of CCL2 release after the injury in response to different pain-inducing factors such as TNFα, which activates release of CCL2 through the c-jun-N-terminal kinase (JNK) pathway (Gao et al. 2009). Astrocytes react also directly on CCL2 presence with the increased expression of GFAP and following activation leads to CCL2 overexpression and to continued recruitment of immune cells or activation of glial cells (especially microglia) which are releasing more pro-inflammatory factors and pain enhancers.

6.5 Functions

Generally, CCL2 is a classic chemoattractant agens released from macrophages, monocytes and dendritic cells and has a function of recruiting macrophages, memory T-cells and dendritic cells to the inflammation site. Additionally, CCL2 and CCR2 expression was found on all the types of nociceptive afferent fibers (non-peptidergic and peptidergic C fibers and Aδ fibers) in DRG and in astrocytes and microglia in of the spinal cord. Increased extracellular concentration of CCL2 cause general sensitization of spinal cord neurons and amplifies nociceptive transmission in the spinal cord. Due to its ability to affect neurons and glial cells CCL2 released in the spinal cord after the injury, induces inflammatory response and modulates pain transmission in the spinal cord and thus participates in the development of pathological states such as mechanical allodynia and thermal hyperalgesia. CCl2 can contribute to pain sensation by different mechanisms. Simultaneously, applying of CCR2 antagonists leads to decreasing of CCL2 pro-inflammatory effects connoting dependence of these mechanisms on CCL2/CCR2 interactions. (Dansereau et al. 2008; Zhu et al. 2014)

In case of spinal cord injury-induced pain expression of CCL2 and its receptor CCR2 was found in astrocytes and neurons respectively. (Zhu et al. 2014) In the spinal cord, CCL2 released from astrocytes can induce heat hyperalgesia through the mechanism of activation of microglial cells (Pevida et al. 2014) which produce pain enhancing factors such as TNFα leading to the increased frequency of spontaneous glutamatergic excitatory postsynaptic currents (EPSC) on neuron synapses within substantia gelatinosa (Huang et al. 2014) or IL-1β causing enhancement of NDMA and AMPA receptors activity (Baamonde, Hidalgo, and Menéndez 2011) Microglia, activated by CCL2, can also participate in the development of cold hyperalgesia. (Pevida et al. 2013)

In DRG, CCL2 and CCR2 are expressed in neurons and macrophages respectively after the spinal cord injury. CCL2 released from the neurons acts as a chemoattractant and recruits macrophages which release pro-inflammatory factor causing pain sensation (Biber and Boddeke 2014). “Inflammatory soup’s” constituents induce hypersensitivity of receptors on sensory neurons through the different mechanisms:

- Production and release of neuropeptides such as CGRP and SP (TNFα, IL-1β, NGF) (Üçeyler, Schäfers, and Sommer 2009)
- Modulation of ion channels activity (TNFα (Czeschik et al. 2008), CCL2 (Sun et al. 2006), IL-1β(Desson and Ferguson 2003))
- Protein-kinase dependent mechanism (IL-1β) (Obreja et al. 2002)

CCL2 potentially stimulates macrophages to produce 4-hydroxy-2-nonenal (4-HNE) –a downstream product of ROS- which induces expression of cyclooxygenase-2 (Cox-2). Cox-2 on the periphery then provides prostaglandins production and following hypersensitivity of TRPV1 and TRPA1 receptors, inducing thermal and mechanical hyperalgesia respectively. (Pflücke et al. 2013)

However, CCL2 not only affects pain processes via macrophages but also can directly influence neurons. Chemokine itself triggers release of neuropeptides like CGRP from DRG neurons via PTX-sensitive G-protein/PLC/PKC pathway (Qin, Wan, and Wang 2005) and also directly modulate neuron sensitivity. It’s been shown, that presence of this chemokine may increase excitability of ion channels lowering threshold for action potential occurring or activating nonselective cationic conductance (NSCC) in a concentration-dependent manner and also able to modulate Ca^{2+} and Na^{+} currents. (Sun et al. 2006) This capability to affect ion channels and it’s co-localization with TRPV1 receptors suggests the role for CCL2 in TRPV1-dependent thermal hyperalgesia. This connection was confirmed by studies (Spicarova et al. 2014) but the exact mechanisms are still need to be explored.

7 Discussion

As we see from the literature reviewed in this thesis, chemokines, especially CCL2, play an important role in the nociceptive signal transmission and its modulation. Having impact on such necessary compartments of the nociceptive pathway as glial cells and afferent

fibers they contribute to inflammation states and general sensitization. Their presence is connected with both development and maintenance of the chronic pain and trough the different mechanisms leads to pathological phenomena like hyperalgesia and allodynia. Last studies reveal some of these mechanisms and suggest possible ways to prevent them. Significant role of the CCL2 in neuropathic and nociceptive pain is evident, which makes this chemokine a novel future target for chronic pain therapy. In neuropathic pain CCL2 can activate CCR2 receptors on nociceptive neuron directly or through the microglial activation. In nociceptive pain CCL2 released from DRG neurons causes inflammatory pain indirectly, through the other mediators or through the direct impact on ion channels. Nevertheless, the certain pathways of CCL2 influence in the modulation processes remain unclear and still need the future investigation.

8 Literature

Articles and review sources

Apkarian, A. Vania, M. Catherine Bushnell, Rolf-Detlef Treede, and Jon-Kar Zubieta. 2005. 'Human Brain Mechanisms of Pain Perception and Regulation in Health and Disease'. *European Journal of Pain (London, England)* 9 (4): 463–84. https://doi.org/10.1016/j.ejpain.2004.11.001.

Baamonde, Ana, Agustín Hidalgo, and Luis Menéndez. 2011. 'Involvement of Glutamate NMDA and AMPA Receptors, Glial Cells and IL-1β in the Spinal Hyperalgesia Evoked by the Chemokine CCL2 in Mice'. *Neuroscience Letters* 502 (3): 178–81. https://doi.org/10.1016/j.neulet.2011.07.038.

Basbaum, Allan I., Diana M. Bautista, Grégory Scherrer, and David Julius. 2009. 'Cellular and Molecular Mechanisms of Pain'. *Cell* 139 (2): 267–84. https://doi.org/10.1016/j.cell.2009.09.028.

Baumann, Nicole, and Danielle Pham-Dinh. 2001. 'Biology of Oligodendrocyte and Myelin in the Mammalian Central Nervous System'. *Physiological Reviews* 81 (2): 871–927. https://doi.org/10.1152/physrev.2001.81.2.871.

Bavencoffe, Alexis, Shao-Rui Chen, and Hui-Lin Pan. 2014. 'Regulation of Nociceptive Transduction and Transmission by Nitric Oxide'. *Vitamins and Hormones* 96: 1–18. https://doi.org/10.1016/B978-0-12-800254-4.00001-5.

Biber, Knut, and Erik Boddeke. 2014. ‘Neuronal CC Chemokines: The Distinct Roles of CCL21 and CCL2 in Neuropathic Pain’. *Frontiers in Cellular Neuroscience* 8: 210. https://doi.org/10.3389/fncel.2014.00210.

Braz, Joao M., Mohammed A. Nassar, John N. Wood, and Allan I. Basbaum. 2005. ‘Parallel “Pain” Pathways Arise from Subpopulations of Primary Afferent Nociceptor’. *Neuron* 47 (6): 787–93. https://doi.org/10.1016/j.neuron.2005.08.015.

Burnstock, Geoffrey. 2006. ‘Historical Review: ATP as a Neurotransmitter’. *Trends in Pharmacological Sciences* 27 (3): 166–76. https://doi.org/10.1016/j.tips.2006.01.005.

Ch Beck, Grietje, Paul Brinkkoetter, Christine Hanusch, Jutta Schulte, Klaus van Ackern, Fokko J van der Woude, and Benito A Yard. 2004. ‘Clinical Review: Immunomodulatory Effects of Dopamine in General Inflammation’. *Critical Care* 8 (6): 485–91. https://doi.org/10.1186/cc2879.

Chen, Long-Ju, Fu-Gui Zhang, Jing Li, Hong-Xian Song, Li-Bing Zhou, Bo-Chun Yao, Feng Li, and Wen-Chun Li. 2010. ‘Expression of Calcitonin Gene-Related Peptide in Anterior and Posterior Horns of the Spinal Cord after Brachial Plexus Injury’. *Journal of Clinical Neuroscience* 17 (1): 87–91. https://doi.org/10.1016/j.jocn.2009.03.042.

Cheng, Wei, Qin Yin, Ming-Yue Cheng, Hong-Sheng Chen, Shu Wang, Tao Feng, Yin-Ming Zeng, and Gong-Jian Liu. 2009. ‘Intracerebroventricular or Intrathecal Injection of Glycine Produces Analgesia in Thermal Nociception and Chemical Nociception via Glycine Receptors’. *European Journal of Pharmacology* 614 (1–3): 44–49. https://doi.org/10.1016/j.ejphar.2009.04.052.

Chizh, B. A., and P. Illes. 2001. ‘P2X Receptors and Nociception’. *Pharmacological Reviews* 53 (4): 553–68.

Czeschik, Johanna Christina, Tim Hagenacker, Maria Schäfers, and Dietrich Büsselberg. 2008. ‘TNF-Alpha Differentially Modulates Ion Channels of Nociceptive Neurons’. *Neuroscience Letters* 434 (3): 293–98. https://doi.org/10.1016/j.neulet.2008.01.070.

Dansereau, Marc-André, Romain-Daniel Gosselin, Michel Pohl, Blandine Pommier, Patricia Mechighel, Annie Mauborgne, William Rostene, et al. 2008. ‘Spinal CCL2 Pronociceptive Action Is No Longer Effective in CCR2 Receptor Antagonist-Treated

Rats'. *Journal of Neurochemistry* 106 (2): 757–69. https://doi.org/10.1111/j.1471-4159.2008.05429.x.

Desson, Sheana E., and Alastair Victor Ferguson. 2003. 'Interleukin 1beta Modulates Rat Subfornical Organ Neurons as a Result of Activation of a Non-Selective Cationic Conductance'. *The Journal of Physiology* 550 (Pt 1): 113–22. https://doi.org/10.1113/jphysiol.2003.041210.

Fields, H. L., A. Malick, and R. Burstein. 1995. 'Dorsal Horn Projection Targets of ON and OFF Cells in the Rostral Ventromedial Medulla'. *Journal of Neurophysiology* 74 (4): 1742–59. https://doi.org/10.1152/jn.1995.74.4.1742.

Gao, Yong-Jing, Ling Zhang, Omar Abdel Samad, Marc R. Suter, Kawasaki Yasuhiko, Zhen-Zhong Xu, Jong-Yeon Park, Anne-Li Lind, Qiufu Ma, and Ru-Rong Ji. 2009. 'JNK-Induced MCP-1 Production in Spinal Cord Astrocytes Contributes to Central Sensitization and Neuropathic Pain'. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 29 (13): 4096–4108. https://doi.org/10.1523/JNEUROSCI.3623-08.2009.

Gold, Michael S, and Gerald F Gebhart. 2010. 'Nociceptor Sensitization in Pain Pathogenesis'. *Nature Medicine* 16 (11): 1248–57. https://doi.org/10.1038/nm.2235.

Griffith, Jason W., Caroline L. Sokol, and Andrew D. Luster. 2014. 'Chemokines and Chemokine Receptors: Positioning Cells for Host Defense and Immunity'. *Annual Review of Immunology* 32 (1): 659–702. https://doi.org/10.1146/annurev-immunol-032713-120145.

Hanani, Menachem. 2005. 'Satellite Glial Cells in Sensory Ganglia: From Form to Function'. *Brain Research Reviews* 48 (3): 457–76. https://doi.org/10.1016/j.brainresrev.2004.09.001.

Heinricher, M. M., I. Tavares, J.L. Leith, and B. M. Lumb. 2009. 'Descending Control of Nociception: Specificity, Recruitment and Plasticity'. *Brain Research Reviews* 60 (1): 214–25. https://doi.org/10.1016/j.brainresrev.2008.12.009.

Horste, Gerd Meyer Zu, Holger Heidenreich, Helmar C. Lehmann, Soldano Ferrone, Hans-Peter Hartung, Heinz Wiendl, and Bernd C. KieseierI. 2010. 'Expression of Antigen Processing and Presenting Molecules by Schwann Cells in Inflammatory Neuropathies'. *Glia* 58 (1): 80–92. https://doi.org/10.1002/glia.20903.

Huang, Chung-Yu, Ying-Ling Chen, Allen H. Li, Juu-Chin Lu, and Hung-Li Wang. 2014. 'Minocycline, a Microglial Inhibitor, Blocks Spinal CCL2-Induced Heat Hyperalgesia and Augmentation of Glutamatergic Transmission in Substantia Gelatinosa Neurons'. *Journal of Neuroinflammation* 11 (January): 7. https://doi.org/10.1186/1742-2094-11-7.

Hyman, Steven E. 2005. 'Neurotransmitters'. *Current Biology* 15 (5): R154–58. https://doi.org/10.1016/j.cub.2005.02.037.

Jeon, Sang-Min, Kyung-Min Lee, and Hee-Jung Cho. 2009. 'Expression of Monocyte Chemoattractant Protein-1 in Rat Dorsal Root Ganglia and Spinal Cord in Experimental Models of Neuropathic Pain'. *Brain Research* 1251 (January): 103–11. https://doi.org/10.1016/j.brainres.2008.11.046.

Ji, Ru-Rong, Temugin Berta, and Maiken Nedergaard. 2013. 'Glia and Pain: Is Chronic Pain a Gliopathy?' *Pain* 154 Suppl 1 (December): S10-28. https://doi.org/10.1016/j.pain.2013.06.022.

Julian, Fred J., and David E. Goldman. 1962. 'The Effects of Mechanical Stimulation on Some Electrical Properties of Axons'. *The Journal of General Physiology* 46 (2): 297–313.

Krames, Elliot S. 2015. 'The Dorsal Root Ganglion in Chronic Pain and as a Target for Neuromodulation: A Review: DRG-A Robust Target for Neuromodulation'. *Neuromodulation: Technology at the Neural Interface* 18 (1): 24–32. https://doi.org/10.1111/ner.12247.

Kwon, Min Jung, Hae Young Shin, Yuexian Cui, Hyosil Kim, Anh Hong Le Thi, Jun Young Choi, Eun Young Kim, Dong Hoon Hwang, and Byung Gon Kim. 2015. 'CCL2 Mediates Neuron-Macrophage Interactions to Drive Proregenerative Macrophage Activation Following Preconditioning Injury'. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 35 (48): 15934–47. https://doi.org/10.1523/JNEUROSCI.1924-15.2015.

Leung, Lawrence, and Catherine M Cahill. 2010. 'TNF-α and Neuropathic Pain - a Review'. *Journal of Neuroinflammation* 7 (April): 27. https://doi.org/10.1186/1742-2094-7-27.

Mayer, Mark L., Ladislav Vyklicky Jr, and John Clements. 1989. ‘Regulation of NMDA Receptor Desensitization in Mouse Hippocampal Neurons by Glycine’. *Nature* 338 (6214): 425–27. https://doi.org/10.1038/338425a0.

McGillis, J. P., M. L. Organist, and D. G. Payan. 1987. ‘Substance P and Immunoregulation.’ *Federation Proceedings* 46 (1): 196–99.

Mendell, Lorne M. 2011. ‘Computational Functions of Neurons and Circuits Signaling Injury: Relationship to Pain Behavior’. *Proceedings of the National Academy of Sciences* 108 (Supplement 3): 15596–601. https://doi.org/10.1073/pnas.1012195108.

Monteclaro, F. S., and I. F. Charo. 1997. ‘The Amino-Terminal Domain of CCR2 Is Both Necessary and Sufficient for High Affinity Binding of Monocyte Chemoattractant Protein 1. Receptor Activation by a Pseudo-Tethered Ligand’. *The Journal of Biological Chemistry* 272 (37): 23186–90.

Obreja, Otilia, Parvinder K. Rathee, Kathrin S. Lips, Carsten Distler, and Michaela Kress. 2002. ‘IL-1 Beta Potentiates Heat-Activated Currents in Rat Sensory Neurons: Involvement of IL-1RI, Tyrosine Kinase, and Protein Kinase C’. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology* 16 (12): 1497–1503. https://doi.org/10.1096/fj.02-0101com.

Olsen, Richard W., and Timothy M. DeLorey. 1999. ‘GABA Receptor Physiology and Pharmacology’. https://www.ncbi.nlm.nih.gov/books/NBK28090/.

Pethő, Gábor, and Peter W. Reeh. 2012. ‘Sensory and Signaling Mechanisms of Bradykinin, Eicosanoids, Platelet-Activating Factor, and Nitric Oxide in Peripheral Nociceptors’. *Physiological Reviews* 92 (4): 1699–1775. https://doi.org/10.1152/physrev.00048.2010.

Pevida, Marta, Sara González-Rodríguez, Ana Lastra, Olivia García-Suárez, Agustín Hidalgo, Luis Menéndez, and Ana Baamonde. 2014. ‘Involvement of Spinal Chemokine CCL2 in the Hyperalgesia Evoked by Bone Cancer in Mice: A Role for Astroglia and Microglia’. *Cellular and Molecular Neurobiology* 34 (1): 143–56. https://doi.org/10.1007/s10571-013-9995-7.

Pevida, Marta, Ana Lastra, Agustín Hidalgo, Ana Baamonde, and Luis Menéndez. 2013. ‘Spinal CCL2 and Microglial Activation Are Involved in Paclitaxel-Evoked Cold

Hyperalgesia'. *Brain Research Bulletin* 95 (June): 21–27. https://doi.org/10.1016/j.brainresbull.2013.03.005.

Pflücke, Diana, Dagmar Hackel, Shaaban A. Mousa, Anna Partheil, Annick Neumann, Alexander Brack, and Heike L. Rittner. 2013. 'The Molecular Link Between C-C-Chemokine Ligand 2-Induced Leukocyte Recruitment and Hyperalgesia'. *The Journal of Pain* 14 (9): 897–910. https://doi.org/10.1016/j.jpain.2013.02.012.

Purves, Dale, George J. Augustine, David Fitzpatrick, Lawrence C. Katz, Anthony-Samuel LaMantia, James O. McNamara, and S. Mark Williams. 2001a. 'Cholinergic Receptors'. https://www.ncbi.nlm.nih.gov/books/NBK10834/.

———. 2001b. 'Glutamate Receptors'. https://www.ncbi.nlm.nih.gov/books/NBK10802/.

Qin, Xiaomei, You Wan, and Xian Wang. 2005. 'CCL2 and CXCL1 Trigger Calcitonin Gene-Related Peptide Release by Exciting Primary Nociceptive Neurons'. *Journal of Neuroscience Research* 82 (1): 51–62. https://doi.org/10.1002/jnr.20612.

Reichert, F., A. Saada, and S. Rotshenker. 1994. 'Peripheral Nerve Injury Induces Schwann Cells to Express Two Macrophage Phenotypes: Phagocytosis and the Galactose-Specific Lectin MAC-2'. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 14 (5 Pt 2): 3231–45.

Rexed, B. 1954. 'A Cytoarchitectonic Atlas of the Spinal Cord in the Cat'. *The Journal of Comparative Neurology* 100 (2): 297–379.

Rollins, Barrett J. 1997. 'Chemokines'. *Blood* 90 (3): 909–28.

Rostène, William, Patrick Kitabgi, and Stéphane Mélik Parsadaniantz. 2007. 'Chemokines: A New Class of Neuromodulator?' *Nature Reviews. Neuroscience* 8 (11): 895–903. https://doi.org/10.1038/nrn2255.

Schwarz, M., H. H. Radeke, K. Resch, and P. Uciechowski. 1997. 'Lymphocyte-Derived Cytokines Induce Sequential Expression of Monocyte- and T Cell-Specific Chemokines in Human Mesangial Cells'. *Kidney International* 52 (6): 1521–31.

Sofroniew, Michael V., and Harry V. Vinters. 2010. 'Astrocytes: Biology and Pathology'. *Acta Neuropathologica* 119 (1): 7–35. https://doi.org/10.1007/s00401-009-0619-8.

Spicarova, Diana, Pavel Adamek, Nataliia Kalynovska, Petra Mrozkova, and Jiri Palecek. 2014. ‘TRPV1 Receptor Inhibition Decreases CCL2-Induced Hyperalgesia’. *Neuropharmacology* 81 (June): 75–84. https://doi.org/10.1016/j.neuropharm.2014.01.041.

Sun, J. H., B. Yang, D. F. Donnelly, C. Ma, and R. H. LaMotte. 2006. ‘MCP-1 Enhances Excitability of Nociceptive Neurons in Chronically Compressed Dorsal Root Ganglia’. *Journal of Neurophysiology* 96 (5): 2189–99. https://doi.org/10.1152/jn.00222.2006.

Sung, Chun-Sung, Chen-Hwan Cherng, Zhi-Hong Wen, Wen-Kuei Chang, Shi-Ying Huang, Shinn-Long Lin, Kwok-Hon Chan, and Chih-Shung Wong. 2012. ‘Minocycline and Fluorocitrate Suppress Spinal Nociceptive Signaling in Intrathecal IL-1β-Induced Thermal Hyperalgesic Rats’. *Glia* 60 (12): 2004–17. https://doi.org/10.1002/glia.22415.

Tsuda, Makoto, Yukari Shigemoto-Mogami, Schuichi Koizumi, Akito Mizokoshi, Shinichi Kohsaka, Michael W. Salter, and Kazuhide Inoue. 2003. ‘P2X4 Receptors Induced in Spinal Microglia Gate Tactile Allodynia after Nerve Injury’. *Nature* 424 (6950): 778–83. https://doi.org/10.1038/nature01786.

Üçeyler, Nurcan, Maria Schäfers, and Claudia Sommer. 2009. ‘Mode of Action of Cytokines on Nociceptive Neurons’. *Experimental Brain Research* 196 (1): 67–78. https://doi.org/10.1007/s00221-009-1755-z.

Walsh, David A., Paul I. Mapp, and Sara Kelly. 2015. ‘Calcitonin Gene-Related Peptide in the Joint: Contributions to Pain and Inflammation’. *British Journal of Clinical Pharmacology* 80 (5): 965–78. https://doi.org/10.1111/bcp.12669.

Wang, Y., T. Summers, W. Peterson, E. Miiller, and B.D. Burrell. 2015. ‘Differential Effects of GABA in Modulating Nociceptive vs. Non-Nociceptive Synapses’. *Neuroscience* 298 (July): 397–409. https://doi.org/10.1016/j.neuroscience.2015.04.040.

White, Fletcher A., Jihu Sun, Stephen M. Waters, Chao Ma, Dongjun Ren, Matthew Ripsch, Jeremy Steflik, Daniel N. Cortright, Robert H. LaMotte, and Richard J. Miller. 2005. ‘Excitatory Monocyte Chemoattractant Protein-1 Signaling Is up-Regulated in Sensory Neurons after Chronic Compression of the Dorsal Root Ganglion’. *Proceedings of the National Academy of Sciences of the United States of America* 102 (39): 14092–97. https://doi.org/10.1073/pnas.0503496102.

Wong, L. M., S. J. Myers, C. L. Tsou, J. Gosling, H. Arai, and I. F. Charo. 1997. 'Organization and Differential Expression of the Human Monocyte Chemoattractant Protein 1 Receptor Gene. Evidence for the Role of the Carboxyl-Terminal Tail in Receptor Trafficking'. *The Journal of Biological Chemistry* 272 (2): 1038–45.

Wu, Fang, Wei Zhang, Lin Li, Fenping Zheng, Xueting Shao, Jiaqiang Zhou, and Hong Li. 2011. 'Inhibitory Effects of Honokiol on Lipopolysaccharide-Induced Cellular Responses and Signaling Events in Human Renal Mesangial Cells'. *European Journal of Pharmacology* 654 (1): 117–21. https://doi.org/10.1016/j.ejphar.2010.11.022.

Zhu, Xiang, Su Cao, Ming-Di Zhu, Jin-Qian Liu, Jun-Jie Chen, and Yong-Jing Gao. 2014. 'Contribution of Chemokine CCL2/CCR2 Signaling in the Dorsal Root Ganglion and Spinal Cord to the Maintenance of Neuropathic Pain in a Rat Model of Lumbar Disc Herniation'. *The Journal of Pain: Official Journal of the American Pain Society* 15 (5): 516–26. https://doi.org/10.1016/j.jpain.2014.01.492.

Zylka, Mark J., Frank L. Rice, and David J. Anderson. 2005. 'Topographically Distinct Epidermal Nociceptive Circuits Revealed by Axonal Tracers Targeted to Mrgprd'. *Neuron* 45 (1): 17–25. https://doi.org/10.1016/j.neuron.2004.12.015.

Internet and books sources

'Elsevier: Gray's Anatomy, 41st Edition: Standring'. n.d. Accessed 3 May 2018. https://www.elsevier.ca/ca/product.jsp?isbn=9780702052309.

'IASP Terminology - IASP'. n.d. Accessed 4 May 2018. https://www.iasp-pain.org/terminology?navItemNumber=576#Pain.

'Miller-Keane Encyclopedia & Dictionary of Medicine, Nursing & Allied Health - 7th Edition'. n.d. Accessed 3 May 2018. https://www.elsevier.com/books/miller-keane-encyclopedia-and-dictionary-of-medicine-nursing-and-allied-health/miller-keane/978-0-7216-9791-8.

National Research Council (US) Committee on Recognition and Alleviation of Pain in Laboratory Animals. 2009. *Recognition and Alleviation of Pain in Laboratory Animals*. The National Academies Collection: Reports Funded by National Institutes of Health. Washington (DC): National Academies Press (US). http://www.ncbi.nlm.nih.gov/books/NBK32658/.

Spinal Cord – Gray Matter (Section) Latina: Medulla Spinalis – Substantia Grisea. Own work. https://commons.wikimedia.org/wiki/File:Medulla_spinalis_-_Substantia_grisea_-_English.svg#file.

'The Basic Types of Pain'. n.d. Www.PainScience.Com. Accessed 3 May 2018. https://www.painscience.com/articles/pain-types.php.