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The physiology and modulation of nociception in mammals

A fájdalomérzékelés élettana és szabályozása emlősökben

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Abstract

The focus of this thesis lies on gaining a deeper understanding of the physiological functions involved in mammalian nociception. This literature review investigates and summarizes the main nociceptive pathways and highlights the foremost relevant structures and substances. Modulatory points, neurotransmitters, different nerve fibers, receptor and pain types are all outlined and explained. Following this recapitulative overview, major modulatory points and alternative ways to reduce pain are emphasized as well. In the future surgeons and veterinarians need to be able to contemporarily influence various points in the nociceptive pathway in order to provide the best possible quality of life of our patients.

Absztrakt

A jelen szakdolgozat témája az emlősök fájdalomérzékelése. Munkámban leírásra kerül minden olyan elem, amely az emlősök nocicepciójában részt vevő élettani funkciók létrehozásában és szabályozásában fontos szerepet tölt be, mint a nociceptív fájdalomérzékelő idegpályák, illetve a fájdalomérzékelés főbb agyi központjai, és az ezt elősegítő specifikus molekulák. A rendszer alapvető elemeinek ismertetését követően a dolgozatban bemutatásra kerül a fájdalomérzetet továbbító idegi jelátviteli rendszer; az érzetet kiváltó neurotranszmitterek, idegrostok illetve ezek azon szinapszisai, ahol a jel befolyásolható; a fájdalmat érzékelő receptorok és maguk a fiziológiás fájdalom típusai is. Ezt követően az orvoslásban használatos fájdalomcsillapító módszerek alapos leírása következik. A jövőben a sebészeknek és állatorvosoknak képesnek kell lenniük arra, hogy a fájdalomérző pálya különböző pontjainak működésébe specifikus módon avatkozhassanak be, így biztosítva a lehető legjobb életminőséget pácienseink számára. A dolgozatban összefoglalt tudásanyag ezt a folyamatot kívánja megkönnyíteni számukra.

Contents

Abbreviations

ACC	Anterior Cingulate Cortex
BDNF	Brain Derived Neurotrophic Factor
BoNTs	Botulinum Neurotoxins
CGRP	Calcitonin Gene-Related Peptide
CNS	Central Nervous System
DBS	Deep Brain Stimulation
DRG	Dorsal Root Ganglion
FNE	Free Nerve Endings
HPA	Hypothalamus-Pituitary-Adrenal Axis
IL	Interleukin
mGLU1	Metabotropic Glutamate Group1 Receptor
MRI	Magnetic Resonance Imaging
NK	Neurokinin
NMDA	N-Methyl-D-Aspartate
PAG	Periaqueductal Gray
PFC	Prefrontal Cortex
PSN	Primary Sensory Neuron
RVM	Rostral Ventromedial Medulla
SGR	Substantia Gelatinosa of Rolando
SIA	Stress Induced Analgesia
SP	Substance P

SSRI	Selective Serotonin Reuptake Inhibitor
STT	Spinothalamic Tract
Symp.	Sympathetic
TCA	Tricyclic Antidepressants
TRP	Transient Receptor Potential Protein
TRPV	Transient Receptor Potential Channel Subfamily V1
VPL	Ventral Posterolateral Nucleus

1 Introduction

The worldwide daily use of pain killing drugs underlines the need for veterinarians to have a deeper understanding of the physiology of mammalian nociception and on how the pain pathways work. At the very core, the nociceptive pathway leading to the sensation of pain severs as a warning. This informs the mammals that there is a harmful stimuli which is ought to be avoided as there is a possible threat to the existence of the given species [1]. This was the case for millions of years and served the evolution of the mammalian kind. Nowadays veterinarians are facing patients with many different origins of pain such as visceral pain or unavoidable post operative pain. We shall in the long term provide the highest possible quality of life with the least amount of side effects. Dampening the chronic pain sensation and limiting acute nociceptive impulses are always of high veterinary importance. This underlines the need of a deeper understanding of the nociceptive pathways as well as the neurotransmitters and receptors involved. While the short-term pain served a protection role the long-term pain has more adverse effects than benefits. More often than not we will see maladaptive behaviors as a result. In addition, we aim to reduce persistent nociceptive and neuropathic pain as this prevents good wound healing and the emotional wellbeing. Throughout mammalian evolution the main nociceptive pathway remained the same [2]. This thesis will focus on the nociceptive neuroaxis pathways and brief focuses are laid on the important aspects, directly or indirectly, connected to these pathways. The gate control theory of pain will not be discussed.

In the first part the ascending and descending pathways are explained. The focus is put on the spinothalamic tract (STT) of the ascending pathway and on how the nociceptive stimuli travels from the peripheral nervous system to the CNS. The location of the first, second and third order neurons are described in connection with the different laminae in the spinal cord. Following this, the descending pathway response mechanisms to nociceptive stimuli are investigated. The often-overlooked classification of pain types, nerve fibers and nociceptors are individually presented with the description of how receptors from impulses after they being affected by external stimuli. After this the spotlight is put onto the diverse functional modulation points, the substantia gelatinosa of Rolando and ended by the elaboration of various possible alternative targets to reduce pain.

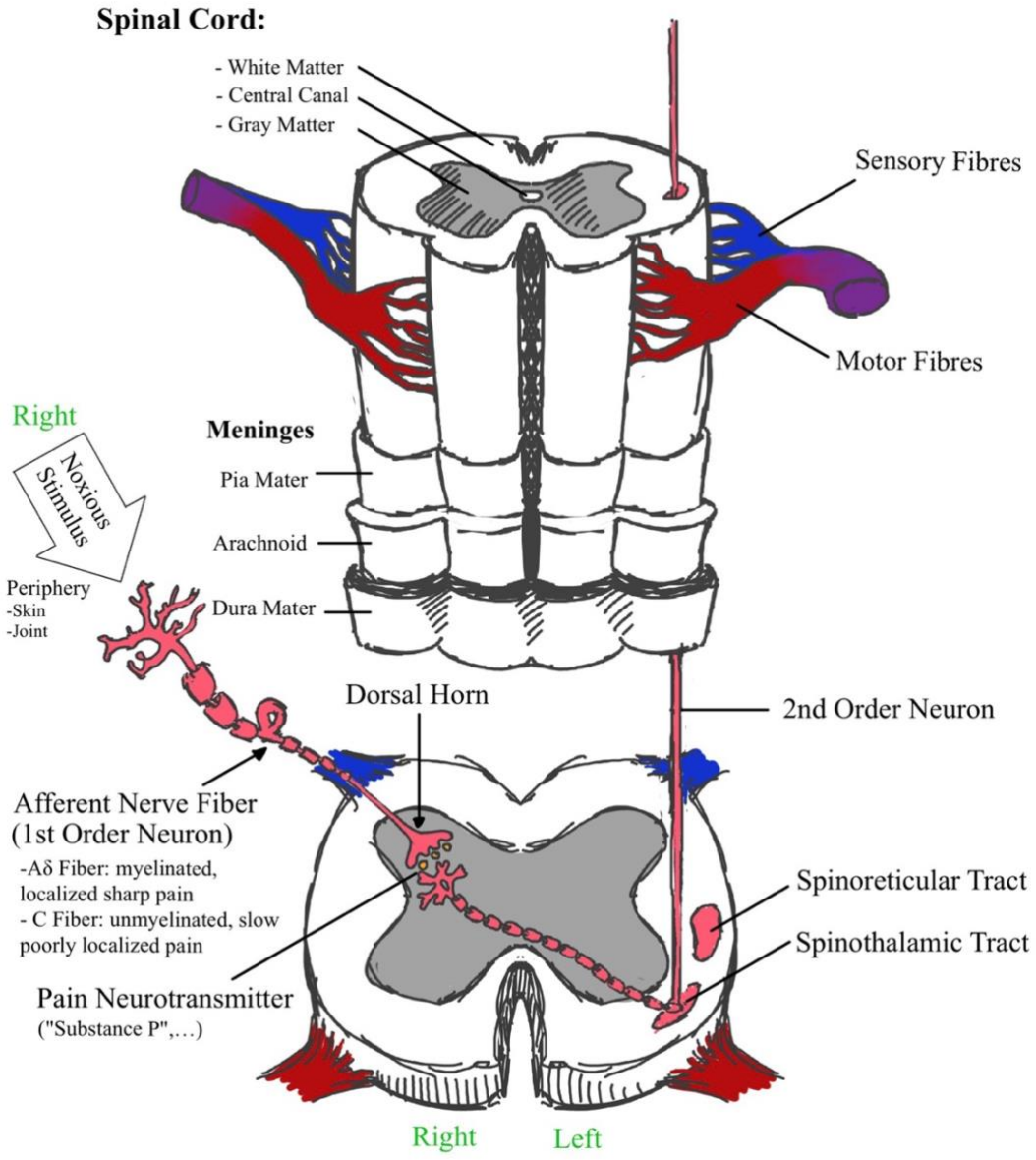
1.1 Goals

The goal of this thesis paper is to get a thorough understanding on how mammalian nociception works and to find the limitations known to man of the available knowledge about this topic in order to pinpoint details and fields of research which will require more in-depth research in the future.

2 Literature review

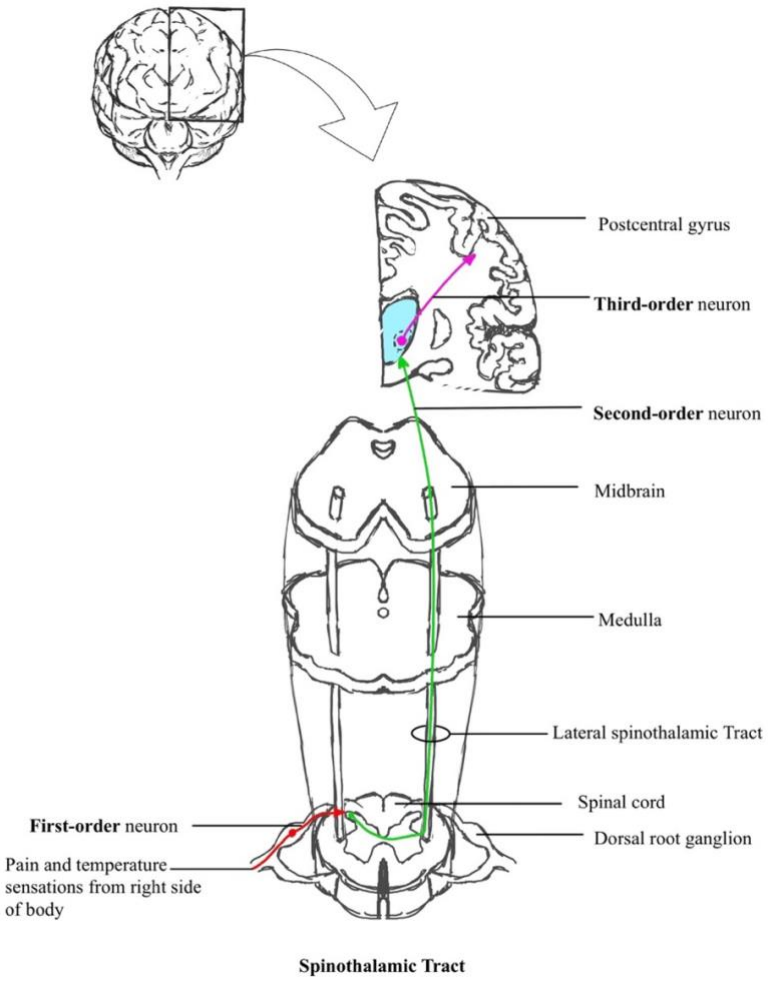
2.1 Pathways of pain: ascending and descending

It is recognized that the nociceptive pathways show a high degree of similarity among the mammalian species *(Figure 1)*. Throughout evolution some species show slight deviations from the central nociceptive but in general we can say that the nociception pathways and signaling mechanisms are highly conserved among mammals [2].



"Figure 1: A hypothetical limb injury with the associated ascending nociceptive pathway [3]".

Nociceptors are biochemically specialized cells which are used to detect internal and externally originating harmful noxious stimuli. These stimuli can by physical, chemical or thermal and are considered as harmful if the survival of the mammal is threatened [4]. The ascending pathway is used to transmit the nociceptive stimuli from the wound to the thalamus and finally to the somatosensory cortex. The somatosensory cortex as this is the area of the brain responsible for the actual generation of the sensation of pain, the memory thereof as well as part of the initiation of the following reflex cascade. Divers factors such as emotions and negative past experiences, stress and fear play a significant role on how individual mammals sense pain [5]. Due to the mixture of physiological and psychological aspects happening in the somatosensory cortex, the upcoming description and explanation is limited to the physiological pathways. The psychologically driven mechanisms of the cortex are excluded.



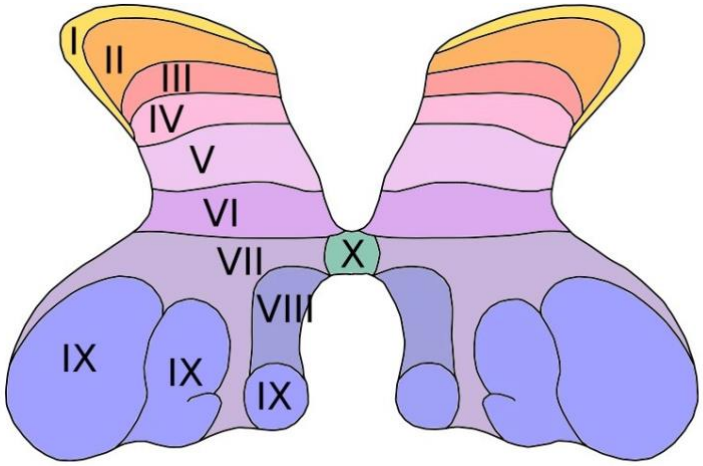
"Figure 2: A visual representation of the first, second and third-order neuron's location within the spinothalamic tract [6]".

The ascending pathway consists of various neuronal pathways but the main one responsible for nociception transmission is the spinothalamic tract (STT). The STT is subdivided in anterior and lateral tract. Our focus will remain with the lateral STT because this one "transport" the noxious information of temperature and various other harmful stimuli from peripherally located nociceptors to the somatosensory regions of the thalamus *(Figure 2).* Sensory information like crude touch and pressure is transmitted by the anterior spinothalamic tract [7].

Within the STT there are three different types of fibers which all have a different role in nociceptive information channeling. Fast and acute nociceptive stimuli transmission happens via myelinated A-delta and type III fibers. Long lasting transmission of slow burning nociceptive stimuli is done mainly by unmyelinated-C fibers and to a smaller extend also by the A-delta fibers. These fibers are also characteristically known to transmit information of poorly localizable pain [6].

Nociceptive messages formed in the FNE are transmitted to the dorsal root ganglion (DRG) via the axons of primary afferent neuron. The enlargement of the primary sensory neurons (PSN) cell body is named as the DRG [8]. The DRG fibers terminate in the brain stem where they synapse with the dorsal column nuclei [9]. After a peripheral nociceptor irritation, the axon of the afferent neuron will transmit the information by encoding the received stimuli in very specific frequency pattern of impulses. The frequency of the afferent neuron is directly proportionate to the severity and to the intensity of the stimuli. The newly generated stimulus travels to the DRG which is embedded in the substance of the dural sheath while being situated within the neuroforamen. This can be found bilaterally in the proximity of every mammalian vertebral junction [10]. The first order neurons are pseudounipolar type neurons [11]. Inside the DRG there is an eight times higher density in glial cells than neurons. A-type-neurons are considered as "large-light neurons" and the C-type-neurons are called "small-dark" neurons. To maintain the independent function of the aforementioned structures each PSN is enveloped by satellite glial cells [10]. The glial cell's most essential function in the mammalian CNS is to allow the fast conduction of information by producing myelin sheets around the axons [12]. From the cell body there is a bilateral bifurcation [10]. The peripheral branch brings afferent information from the FNE in direction of the DRG and the central extension will enter the spinal cord. Pseudounipolar neurons from the DRG represent the first order neurons of the spinothalamic tract. The exact entry point of the first order neurons into the spinal cord is the lateral dorsal root entry zone [7]. After which the

first order neurons will become part of the Lissauer tract by giving rise to ascending and descending branches. Within the Lissauer's tract the axon branches run cranially or caudally for one or two spinal cord segments before entering the gray matter of the dorsal horn [13]. Within the gray matter we differentiate 10 different zones which are organized in lamellar scheme *(Figure 3)*. These are termed as Rexed laminae. We focus on the more dorsally lying laminae I, II and IV as they are involved with axons that transmit nociceptive information. Interestingly the total amount visceral motor neurons are situated within the laminae VII. Each of the lamina have a slightly different cytoarchitecture [14].



"Figure 3: Image showing the cross section of the spinal cord with the 10 different laminae layers of the gray matter [15]. The Swedish neuroscientist Bror Rexed identified and assigned these laminae [16]".

In the further course of the ascending nociceptive pathway the first order neurons will synapse with second-order neurons in the gray matter. The second order neurons will decussate in the anterior white commissure of the spinal cord and then keep ascending to the CNS in the contralateral lateral spinothalamic tract [17]. Within the gray matter the first to second-order neuron synaptic site structure is termed as "substantia gelatinosa of Rolando" (SGR). Located dorsally on the spinal cord this neuroanatomical region plays a significant role in the modulation and transmission of nociceptive stimuli and runs all the way along the spinal cord at the level Rexed lamina II height of the gray matter [18]. Neurotransmitters are required for nociceptive information transmission from the primary order neuron to the secondary order neuron. Substance P, glutamate, vasoactive peptide, somatostatin and calcitonin gene-related peptide (CGRP) are nociceptive neuron activating substances. They act within the spinal cord and cause a release of glutamate. This starts the "central

sensitization" process as the N-methyl-D-aspartate (NMDA) receptors become more sensitive to glutamate and increased amount of glutamate also activates the neurons by increasing the sensitivity and thus the excitability [14].

The second order neuron's afferent branches run within the Lissauer's tract all the way cranially until they reach the thalamus [13]. The main part of the dorsolateral STT are axons which come from the contralateral lamina I of the of the

spinal cord. A significantly smaller portion arises from the deeper spinal cord laminae. The axons of the laminae IV, V and VII-X compose the ventral spinothalamic tract [19]. The anterior STT is also used for ascending transmission of nociceptive stimuli of temperature and touch [14]. Proprioception and vibration information is transmitted via the ascending sensory tract [19]. The lateral spinothalamic tract and the anterior spinothalamic tract stay separated while rising up the spinal cranially cord towards the thalamus. During the remaining ascent towards the brain the STT remains within the white matter of the spinal cord [7]. In the medulla the "spinal lemniscus" is formed which is the fusion of the lateral and anterior spinothalamic tract with the spinotectal pathway [7]. The spinotectal tract starts in the spinal cord and runs towards various structures in the midbrain. The projections serve for pain processing and the muscular reflexes following a nociceptive stimuli [20]. Other termination sites for the ascending second-order axons are the intralaminar nucleus of the thalamus, the reticular formation of the brainstem, the midbrain's periaqueductal gray (PAG) or the ventral posterolateral nucleus (VPL) of the thalamus [14]. Tertiary axons leaving the thalamus run through the internal capsule and end up in the posterior paracentral lobule of the parietal lobe and the postcentral gyrus of the brain. The connection from the second order neuron to the third order neuron happens in the ventral posterolateral nucleus of the thalamus [14]. The internal capsule is a white matter structure which can be subdivided into anterior and posterior limb and genu. Laterally the internal capsule is bordered by the lentiform nucleus and medially by the thalamus. The function is to link the spinal cord, brainstem and thalamus to the cerebral hemispheres and subcortical structures via ascending and descending fiber tracts [21]. The aforementioned structures are considered as the primary cortical areas to receive nociceptive information. The postcentral gyrus gives rise to the primary somatosensory cortex. In mammals the accurate localization of nociceptive stimuli is based on the so-called somatosensory map [14]. This is a neurological map which organizes different body areas to different parts of the cortex. This neurological map is also called "sensory homunculus" [5]. This concept is conserved among the mammalian species. The processes of the intralaminar nuclei, so the nerve cells located within the internal medullary lamina, reach the rostral cingulate gyrus and the insula. This domain gets the nociceptive stimuli associated with deep or dull pain sensation. Accordingly, this zone plays a role in the long lasting, poorly localizable nociceptive information. Psychological and emotional factors influencing nociception are also linked to the cingulate gyrus and insula [14].

Afferent nociceptive stimuli processing requires numerous receptor types. Signals from the STT will trigger a release of norepinephrine from the neurons of the locus coeruleus which projects to the thalamus. The released norepinephrine will transfer the nociceptive information to the hippocampus, hypothalamus and somatosensory cortex and therefore contributes strongly to the nociception processing in the subcortical and cortical brain area [14]. The hippocampus plays part in learning and memory of nociceptive stimuli [22]. The locus coeruleus is situated dorsolaterally in the brainstem's pons and plays a crucial noradrenergic role [23]. Direct or indirect fiber projections from the nucleus are used to control many autonomic brain functions. These projections run to the amygdala, the rostroventral medulla, to the caudal raphe and to various nuclei like the paraventricular, salivatory and the dorsal motor nucleus of the vagus [24]. However, there are some afferent fibers which do not synapse in the thalamus and run through the given nuclei and these nuclei are then termed "bypass nuclei". Other fibers bypass the entire thalamus and run directly to the somatosensory cortex via a direct spino-cortical circuit. These neurons are called "spino-cortical recipient neurons" [25]. The brain's biggest relay station is located centrally within the skull, and this is the thalamus. All sensory pathways need to pass through this converging structure before the information is transmitted to the cerebral cortex. The thalamus itself has many subdivisions and can be categorized by anatomical location as well as by the related function of the given nuclei. There are 3 main categories of nuclei in the thalamus. The first one being the relay nuclei composed of the later, medial and anterior nuclear group. The lateral nuclear group contains the VPM and the VPL nucleus which are of importance regarding nociceptive information handling. Then there is the reticular nucleus and the intralaminar nuclei. The intralaminar nucleus also has projections to the cortex [26]. From the thalamus the information will continue to the "cortical columns" which are the basic functional units for cerebral cortical processing. Here the nociceptive information will be transformed to a painful sensation [27]. Within the mammalian brain the cortical columns are the most complex structure as they are individual functional units able to process the impulse information [28]. Despite a high level of anatomical knowledge on these cortical modules, currently we still have to accept the columnar organization hypothesis as there is a lack of knowledge and deeper understanding on how these mammalian columns actually function and process the information [29].

2.2 The descending pain pathway

Besides the ascending pathway is also a descending motor pathway which will allow the withdrawal of the limb or other extremity from the source which is harmful to the mammalian organism. The dorsal column and the medial lemniscus pathway will not be covered in this paper as the information channeled is mainly sensory information about proprioception, fine touch and vibration [30].

The main structures involved in the inhibitory descending pathway are the lateral part of the ventrolateral medulla (VLM), the rostral ventromedial medulla and the PAG. The members of the endogenous nociceptive control system are interlinked in a complex network. Within the VLM the alpha-2-adrenoreceptors are responsible for the analgesic effect [31]. The rostral ventral medulla–PAG pathway is considered of the most significant modulation points in the descending pathway. Involved structures are the amygdala and the hypothalamus which are both stress sensitive. The RVM has opioid and cannabinoid receptors which play a significant role in stress induced analgesia (SIA). This works based on the as the hypothalamus ability to release endogenous opioids. Endogenous cannabinoids are broken down with fatty-acid amide hydrolase. Blockage of the this enzyme results in a positive anti-nociceptive effect as the endocannabinoids will be on the receptors for a longer time and in a higher concertation [32]. Recent studies show that imbalances in this pathway facilitate the development and maintenance of chronic pain sensation but also provide us with new possible therapy approaches. The primary relay center and connection point of the descending pathway and the primary afferent fibers happens in the dorsal horn. In this localization we can also find 7 various subtypes of 5-HT receptors which modulate nociceptive information. Within these receptors there are further 14 district serotonin receptor subtypes. They are either ligand gated or G protein-coupled transmembrane receptors. Interestingly it has been proven that the 5-HT4 receptors are not part of the direct opioidergic system in the spinal cord but that the major analgesic role of these receptors is in the descending noradrenergic pathway. A widely used way to provide analgesic care to our patients is the application of selective serotonin reuptake inhibitors (SSRI) or tricyclic antidepressants (TCA). These will increase the serotonin levels and therefore effect the nociceptive 5-HT system [33]. Allodynic pain is triggered by a stimuli which does normally not cause the nociceptive system to activate [34]. The analgesic effect of the 5-HT-7 receptors is suggested to happen due to the activation of the inhibitory GABAergic interneurons located in the spinal cord's dorsal horn. GABA-b and opioid receptor

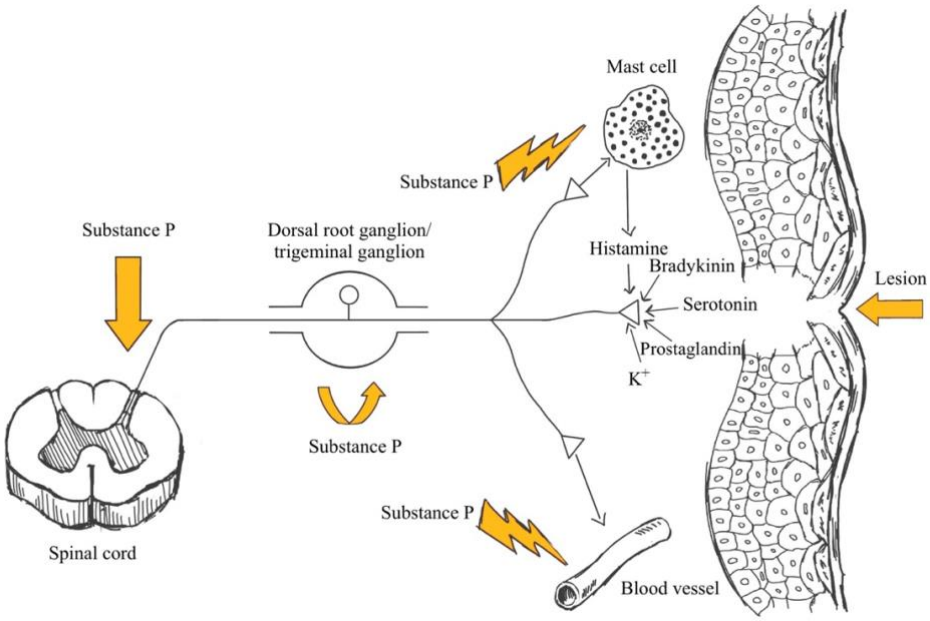
antagonists have been proven to be able to block the 5-HT7 receptors, but the GABA-a antagonists can block these receptors. Concludingly we can say that the descending noradrenergic neurons of the GABAergic pathway elevate the function of the opiate neurons thus leading to a reduced firing rate of the projection neurons towards the thalamus [33].

3 Types of pain: functional, inflammatory, neurogenic

3.1 Functional pain

Functional nociception means that there are nociceptive stimuli that reach the brain without us veterinarians being able to visually localize the organic starting point of the stimuli as there is no need for a macroscopic lesion. Examples highlighting this obstacle are patients with visceral pain or musculoskeletal pain. Many practicing veterinarians do not take this into consideration, but functional pain can be considered as a benign disease and leads to patient distress and a direct reduction of the patient's life quality. To regain their quality of life TCA and SNRI are applied nowadays [35]. Diverse types of nerve damage can lead to functional pain. Physical or chemical nerve insult results in the neuralgia syndrome which means that the mammal will perceive a sensation of a noxious stimuli and sense pain even though no free nerve ending is actively being stimulated. The mechanism behind this peculiar phenomenon is still unclear and will hopefully be available in the coming few years. Nowadays we accept the cortical remapping theory as being at the origin of the phantom pain. In the veterinary field currently applied phantom pain treatment drugs are gabapentin and pregabalin. Opioids are beneficial in phantom pain management as well because they can decrease cortical somatosensory reorganization. Nowadays it is believed that there is a strong correlation between the somatosensory reorganization and the development of the phantom pain syndrome. Antiseizure drugs decrease the severity of the symptoms as the neuropathic pain intensity can be reduced [36].

3.2 Inflammatory pain and the effect of histamine regarding pain



"Figure 4: Illustration of the inflammatory based effects after an external lesion [4]."

In many cases nociception is the result of inflammatory reactions which involve many different molecular mechanisms. This can be due to noxious chemical, thermal or mechanical stimuli. There is a high level of analogy regarding the process and molecules, receptors, transmitters and channels involved all throughout the body [4]. Inflammatory mediators play an important role in the peripheral sensitization and in pain intensity. First there is wide variety of cells which can produce inflammatory mediators, and these will activate phospholipase and protein kinase after having reacted with the nociceptive sensory neurons. Besides immune cells such as lymphocytes, neutrophils and monocytes, keratinocytes and mast cells secrete such inflammatory mediators [37]. Ion channels and receptors are regulated by theses secondary messengers. The noxious stimuli will induce ion channels to open and consequently within the sensory neurons there is an action potential propagation [4]. Vasodilation inducing mediators such as prostaglandins, bradykinins and histamine increase the permeability of the blood vessels, relax arteriolar smooth muscles and contribute to the inflammation increase *(Figure 4)*. The vasoactive substances cause sensitization of the FNE [38]. The aforementioned substances trigger the release of the neuropeptide "substance P" (SP) from the sensory nerves [39]. The production site of SP is in the dorsal root and trigeminal ganglia within the cell bodies of the capsaicin sensitive

sensory peripheral neurons. Prostaglandins, histamine, allergens and leukotrienes all sensitize the peripheral free nerve endings [39]. Substance P carries a vital role in the transmission of nociceptive information in the spinal cord [4]. Up to 80% of the dorsal root ganglia produced SP goes to the peripherally located neuronal branches. Angiotensin converting enzyme and the neutral endopeptidase will cause the breakdown of the SP [4].

3.3 Neurogenic pain

Neurogenic pain is considered a special type of pain because there is no direct nociceptive stimulation originating from disease or injury but there is a constant dysfunction of the peripheral or central nervous system. Synonyms used to describe this condition are: central pain, deafferentation pain, and neurogenic pain. In neurogenic pain the trigger is injury or damage to the somatosensory nervous system. Neurogenic pain patients have a malfunction of the peripheral or central nervous system. Besides the dominant role of SP there are other proinflammatory neuropeptides such as calcitonin gene-related peptide (CGRP) and neurokinin A and B. Anti-inflammatory neuropeptides such as somatostatin and galanin can also be located in the capsaicin sensitive neurons [40]. Vanilloid receptor 1 (VR1) located on small diameter sensory fibers can be opened by thermal stimuli, capsaicin and protons resulting in an increase of the Ca^{2+} inflow. Opening of the Ca^{2+} cation channels happen due to a voltage change triggered by a sodium induced depolarization. The SP release from sensory nerve endings will happen via many different mechanisms and has the tendency to increase its own axonal release. The protein kinase C can be triggered by the bradykinin activation of the B2 receptors. Prostaglandins and inflamed tissue will cause a decrease in the threshold of the sensory neurons. Bradykinin and capsaicin also increase the release of SP [4]. Tropomyosin receptor kinase (TRK) normally play a role in the neuron differentiation, synapse plasticity as well as axonal and dendritic formation [41]. Sensitization of the nociceptors, leading to neurogenic pain can also happen if the high affinity TrkA receptors and the NGF form a NGF/TrkA complex. There are three types of G protein-coupled NK (neurokinin) receptors NK1, NK2 and NK3. SP has the main effect on the NK1 tachykinin receptor. The stimulation of which will increase the intracellular calcium by means of activation of the phospholipase C intracellular inositol and the 1,4,5-triphosphate inositol pathway. Multiplication of the SP release from the axons also happens via increased formation of prostaglandin E2 and increased proliferation of COX2. This happens via the P38 and ERK 2 mitogen activated protein. One can clearly say that the SP

causes a chain reaction as SP receptors can be found on many different proinflammatory cells such as on macrophages, mastocytes, lymphocytes and granulocytes. After the receptor-neuropeptide bond has happened the mastocytes will release histamine and macrophages will launch the production and release of thromboxane, PGE2, TNF and proinflammatory IL-1 and IL-6. Inflammatory and pro-inflammatory molecules directly or indirectly activate and sensitize the nociceptors in the inflammation-damaged tissue. Even the surrounding tissues can suffer from secondary hyperalgesia as the inflammatory molecules have the ability to diffuse and activate additional nearby nociceptors. The majority of the neurogenic inflammation is carried by the SP [4]. NGF is a neurotrophic factor and contributes to the correct healing of various wound types and thus could in theory, if present in a high enough concentration, fight the formation and maintenance of neurogenic pain [42].

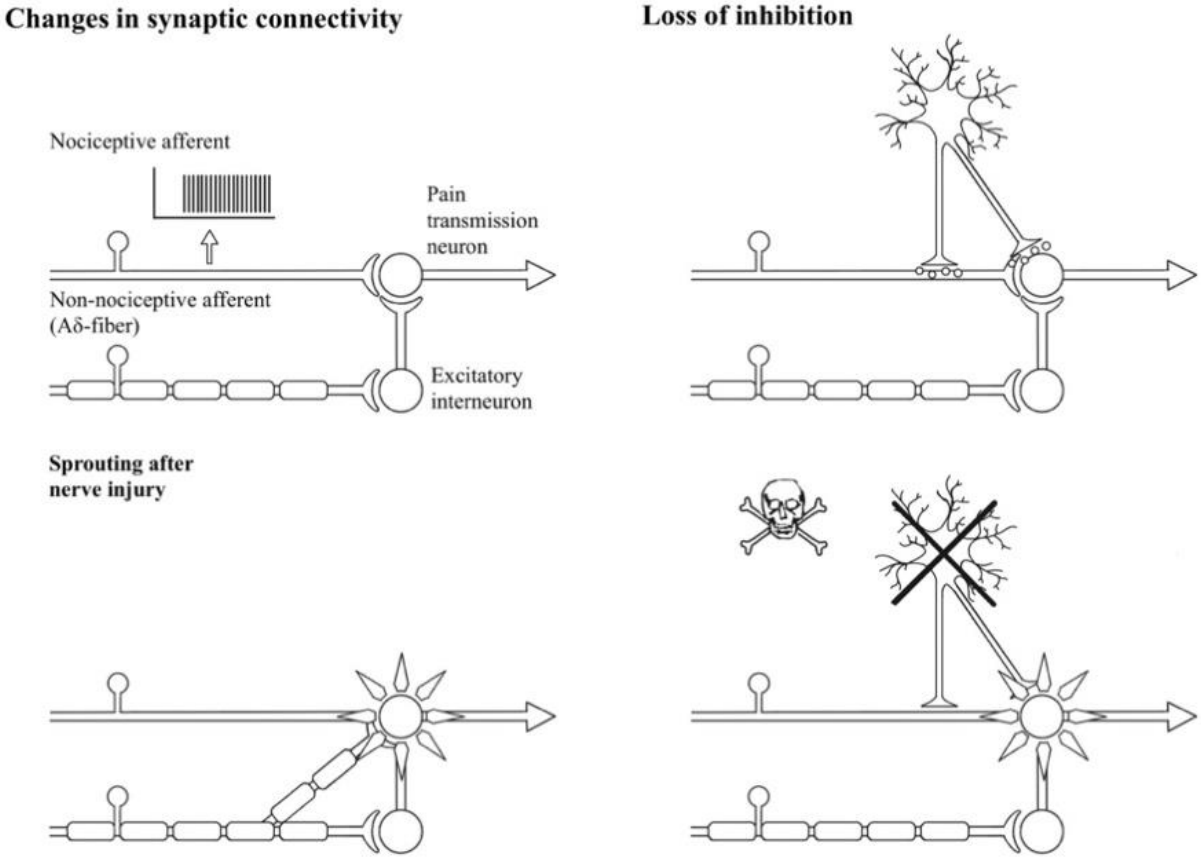
4 Different types of nerve fibers and nociceptive receptors

"Table 1: Summary table of the different fiber types with their associated properties [43]".

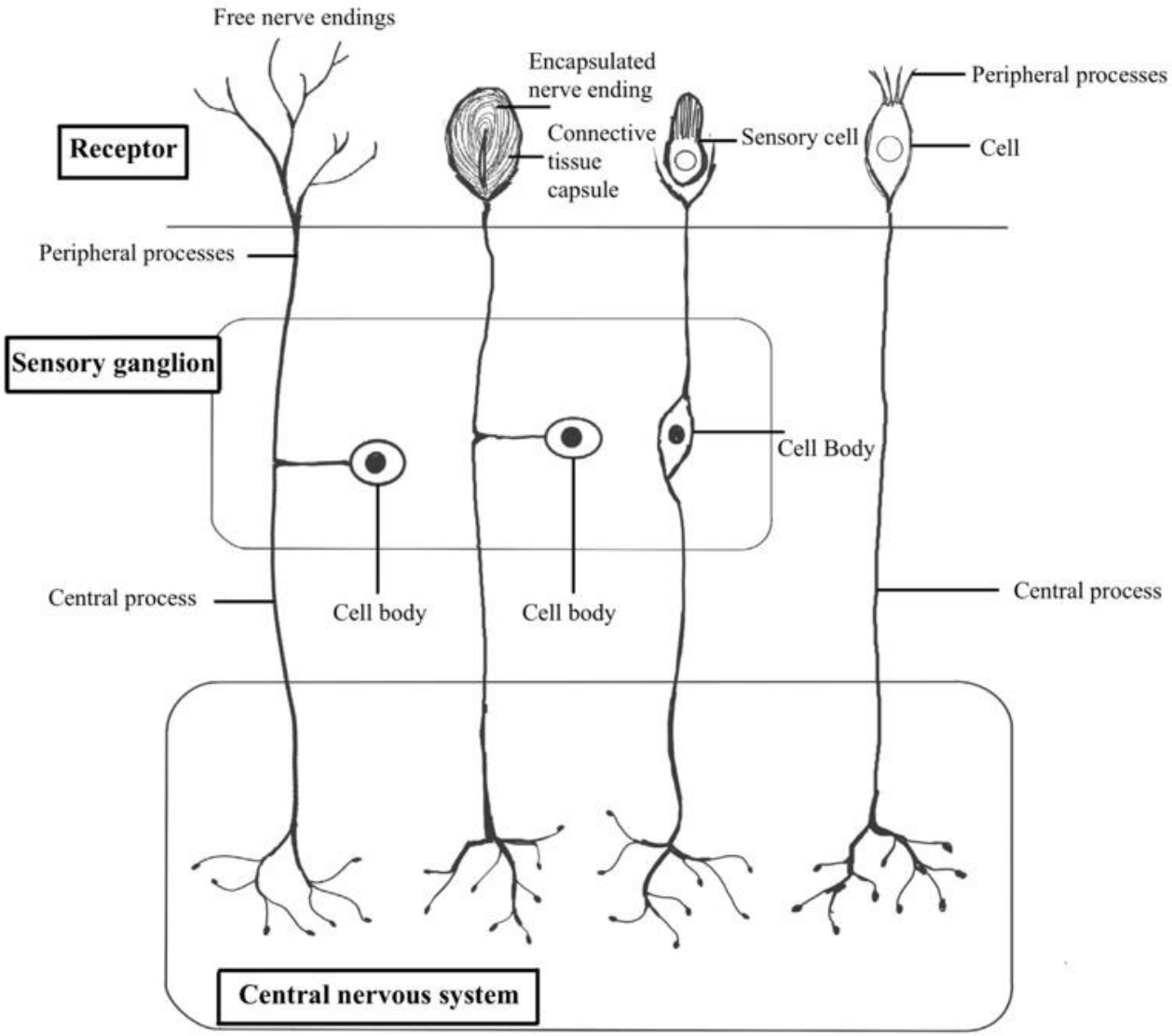
Fiber type	Myelin sheath	Diameter (µM)	Conduction speed (m/second)	Function	Modality	Sensitivity to Lidocaine
Aα	Yes	13-20	80-120	motoric / proprioceptive	-muscles -joints	+
Aβ	Yes	6-12	35-90	pressure mechanoreceptor	-touch -pressure	++
Aδ	Yes	1-5	5-40	nociception / mechanoreceptor	-thermal -touch	+++
C - sympathetic	No	0.3-1.3	0.7-1.3	nociception / touch	-thermal -nociception	++++
C – (dorsal horn)	No	0.4-1.2	0.1-2.0	nociception / touch	-thermal -slow pain	++++

In the mammalian body there are different types of nociceptive fibers and receptors. There are myelinated and unmyelinated nerve fibers. The myelinated Aδ are responsible for the well localized and fast pain transmission to the CNS while the unmyelinated C-fibers are considered in case of slow pain where the nociceptive trigger cannot be localized precisely *(Table 1)*. The slow mammalian pain is linked to an increase of pain perception over time. Throughout the body and especially in the periphery the free nerve endings of the primary afferent nerve fibers form the nociceptors [44]. But besides the afferent nociceptors there are also interneurons which form modulation points and can be situated in the laminae II and III of the spinal cord as well as the projection neurons in the CNS [45]. Projection neurons arising from the neuronal cell body have axons which reach distant regions in the CNS [46]. Interneurons are grouped into excitatory which are glutamate and inhibitory GABA/glycine

operating interneurons [45]. Excitatory interneurons arising from a non-nociceptive A-beta fiber will cause an increase in the frequency and firing rate of the nociceptive signal ultimately leading to a stronger sensation of pain. Nerve injury leads to a similar phenomenon as in the healing process the excitatory interneuron can be bypassed consequently leading to a constant firing or there can be a loss of the inhibitory interneuron function *(Figure 5)*. This may end up in a chronic type of mammalian pain [45].



"Figure 5: Illustration of the passage of an afferent noxious stimuli in the context of different interneuron functional states [47]".



"Figure 6: Simplified illustration of the different types of receptive free nerve endings found in mammals [48]".

The group III fibers have Schwann cells which aid the myelinated fiber formation. The mechanical threshold of the FNE is partly determined by the location and the surrounding tissue type. We may find low-threshold sensory endings in fat cells, thin collagenous layers and in fibroblast composed loose connective tissue. Afferent nociceptors with a high-threshold are located in ligaments, fibrous capsular tissue or in dense collagenous tissue *(Figure 6)* [49]. Diverse encapsulated free nerve endings serve the purpose of fine touch and vibration information capturing [50]. For more accurate information localization the mammalian skin surface is divided into segments. Theses discrete segments are called "dermatomes" and correlate directly to specific levels of the spinal cord. They may or may

not overlap and the clinical relevance of these structures is that they help us to identify the spinal cord lesions or the nerve root compression sites while we perform a neurological physical examination [5].

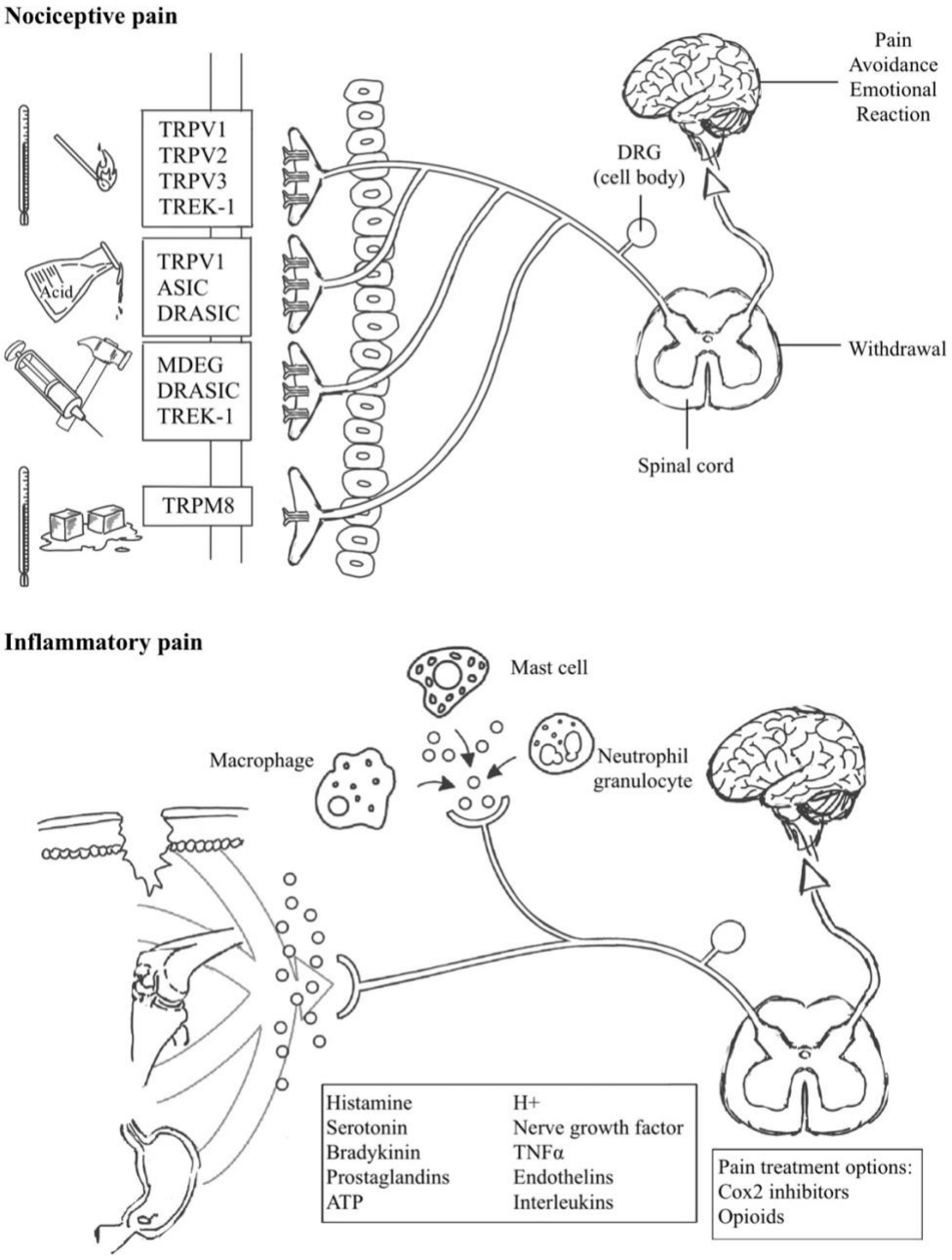
One must underline that the mammalian receptors are not a fix and rigid structure but that their functional threshold can be altered. The term of "central sensitization" means that the excitability of the dorsal horn nociceptive information transmitting neurons is increased for a short time via locally lowering the activation threshold. This happens by means of intracellular protein kinase C and protein kinase A activation. In return this triggers the phosphorylation of the ion channels. Glutamate acting on the NMDA receptors, BDNF acting on the TrkB receptors and SP increases the intracellular Ca^{2+} in the neurons of the dorsal horn [47]. In addition, TRPV1 channels can be found all throughout the body such as in the gastrointestinal, respiratory tract and the heart. Ethanol, capsaicin, NGF, acid and heat can initiate the phosphorylation and thus cause activation of these channels [51]. The long-term neuronal changes within the dorsal horn happen because of transcriptional factors such as JNK, PKA and p38. P38 is a mitogen activated protein kinase which in the presence of substance P will phosphorylate and thus increase the IL-6 expression leading to neurogenic inflammation [52]. The result of the enhanced gene expression and the neurogenic inflammation results is an increased amount of PGE2 thus directly increasing the synaptic excitability [47].

5 Receptors and how they are affected by external stimuli and how the impulse travels therefrom

Traumatic tissue events such as a hernia, bone integrity damage, chemical or physical tissue irritation will have an effect on the receptors of the given region [53]. Unspecialized free nerve endings which start the nociception cascade are termed as nociceptors. The free nerve endings from the first order neurons encode the noxious stimuli in form of an afferent receptor potential which will run along the first order neuron to the spinal cord [54]. Sensory receptors reacting to noxious stimuli are categorized based on fiber type and their given response. The three main harmful stimuli are thermal, mechanical and chemical. Combinations of the indicated are also possible. Within the skin we differentiate high-threshold mechano-nociceptors, A-fiber mechano-heat and C-fiber mechano-heat nociceptors. Additionally, there are polymodal C-fibers which are chemo-sensitive. “Silent” or mechano-insensitive nociceptors are stand out in a way because they are located in almost all tissue and need a long noxious stimuli such as an inflammation to become mechanosensitive [55]. Sensitization means a decrease in the activational threshold and concurrently a stronger response regarding the resulting frequency and magnitude. Diverse inflammatory mediators increase the sensitivity by changing the threshold to noxious stimuli in the receptive field. The nociceptor groups found in various different tissues are nowadays classified based on the receptors and channels that are expressed on the surface such as CGRP and SP as peptigenic receptors, trkA, NGF and P2X3 as purigenic type receptors [56]. Aδ fibers of myelinated axons are able to transmit the action potential at 20 m/s while C-fibers of unmyelinated axons only have a conduction speed of about 2 m/s [54]. Around the C-fibers there are Schwann cells which will increase the conduction speed by myelination [57]. An action potential is only formed if the noxious stimulus surpasses a certain threshold. The thermal receptors are classified into non-nociceptive and nociceptive thermoreceptors. Interestingly the non-nociceptive thermoreceptors discharge at a maximum rate even if the stimulus is still under the painful temperature range [54]. A thermoreceptor triggering stimuli is temperature above 40 degrees Celsius [57]. In general, the nociceptors are electrically inactive and once the activation threshold is reached, always respond with an all-or none action potential [57]. Noxious stimuli activate the TRP channels which are located on the free nerve endings. TRP channel activation will cause a depolarization of the first order neuron and this will force the neuron to fire action potentials. The amplitude of the action potential does not change but it’s the action potential frequency which dictates the stimulus

intensity. Glutamate is an excitatory neurotransmitter used in transmission of information from A-delta fibers to the second order neurons [58].

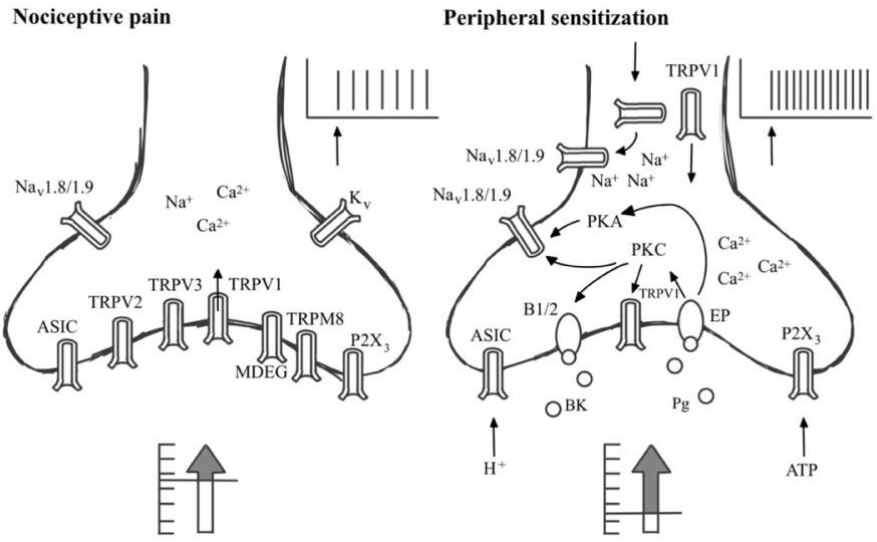
Looking into an example of the cascade of a skin lesion. The first order neuron's free nerve endings are found between the cells of all 4 layers of the epidermis. These intraepidermal nociceptors are needed for various types of cutaneous nociception and pruritus information generation. There is a "synaptic like" contact between the keratinocytes and the FNE. This type of "cell-to-cell connection" is essential for mechanical input to be transformed to an action potential. External stimuli will activate the influx of Ca^{2+} into the keratinocytes. As a result of this there is an outflow of ATP which will trigger and excite the sensory free nerve ending after it bound to the cell's P2 receptor. Mammalian nociceptors are highly controlled by various transient receptor potential proteins (TRP). Within the group of the TRP protein channels we differentiate vanilloid, ankyrin and canonical channels as well as a channel called "no mechanoreceptor potential C" which is encoded by the nompC gene [59]. These broadly distributed non-selective ion channels can activate the nociceptors by a causing a depolarization which generates an action potential. Besides Ca^{2+} depolarization happens via Mg^{2+}, Na^{+} or K^{+} influx [59]. When ion channels open the inflow of these positive ions changes the membrane potential. The membrane potential becomes more positive and this is what we call "depolarization" [57]. P2X4 is a type of P2 receptors. The purinergic P2 receptors are categorized into G-protein coupled P2Y receptors as well as ionotropic P2X receptors. In diseased tissue the ATP-gated P2X bind inflammatory mediator after binding ATP. P2X4 can be stimulated by intra-lysosomal ATP and pH changes [60]. These purinergic P type receptors bind purine-containing molecules and as ATP contains high amount of purine the non-selective ion channel will allow Ca^{2+} inflow [61].



"Figure 7: An illustration of the mammalian nocisensors and their effective domains [47]".

Mammalian "nocisensors" are subdivided into four different types of free nerve endings which are used to differentiate various types of externally or internally originating noxious stimuli. The different free axons endings (FNE) are linked to one of the 4 specific noxious stimuli such as cold, heat, chemical or physical damage while TRPV1 can transmit thermal and chemical informational stimuli *(Figure 7)*. TRPV3 are used solely for heat and TREK-1 are able to transmit thermal as well as cold noxious stimuli [47]. TREK-1 is a double pored potassium channel which can be found all throughout the CNS [62]. These potassium

channels govern the membrane potential excitability under the depolarization threshold [63]. To achieve the highest possible axonal firing rate a long exposure time is needed. The long-term burning sensation is transmitted by the myosin heavy chain A-type I and the C- type fibers [57]. The nociceptor firing speed is influenced by heat activated TRPV channels due to the increase of intracellular Ca^{2+} [59]. DRASIC channels are dorsal root acid sensing ion channels [64]. Acid sensing ion channels (ASIC), TRPV 1 and DRASIC cation channels play an important role in the cutaneous stimuli such as physical and pH changes on the skin [65]. These ion channels belong to the degenerin/epithelial NA+ channels (DEG/ENaC) family [65]. These sodium ion channels can be found in many different mammalian orders and they have a really wide range of functional importance [66]. ASIC are located in the peripheral and central nervous system. These cation channels are proton gated and extracellular pH changes will initiate the activation [67]. Besides the DRASIC and the TREK-1, there are MDEG receptors. These MDEG receptors are fundamentally speaking amiloride-sensitive cationic ion channels which are penetrable by K+, NA+ and Li+ *(Figure 8)* [68]. TRPM8 is a transient receptor potential ion channel used for thermal sensation. At moderately low temperature these ion channels provide an analgesic effect but at really low temperatures the cold-induced noxious stimuli will cause the mammal to sense pain [69]. Animal models lacking the TRPM8 demonstrated that the low temperature analgesic effect is no longer present meaning that the analgesic effect is directly linked to the presence of the TRPM8 receptors albeit the precise location of action are still unknown nowadays [57].



"Figure 8: A representation of a nociceptor and the channels involved in the noxious stimuli initiation [47]".

Let's shortly put the focus on the inflammation mediated sensitization in the spinal cord and how it is linked to mammals sensing chronic pain. Microglial are present in high number in the CNS and play a key role in spinal cord central sensitization process as well as having macrophagic activity [70]. Between the first order neuron and the second order neuron in the spinal cord's dorsal horn there is a loop mechanism of immune cells and their mediators which lead to the "central sensitization". First order nociceptive neurons liberate ATP, CGRP, growth factors such as CSF-1, various cytokines such as CCL2, or TNF-alpha and enzymes such as caspade-6 [71]. CSF stands for "colony stimulating factor" [72]. The release of the inflammatory mediators from the central nerve terminal will force the microglial cells to their "on mode" and increase the production of pro-inflammatory mediators. The cytokines CX3CL1 have a microglial neuronal modulatory effect and are responsible to cause reduced synaptic plasticity [73]. Similarly to the macrophages found all over the mammalian body the microglial produce IL-1beta, PGE2, TNF-alpha as well as BDNF and various other neurotrophins. Accumulation of these factors influences the first order neurons and the second order neurons and underlines the central sensitization process. Chronic pain allows the T-cells to get into the dorsal horn of the spinal cord [71]. Animal models revealed that the phenotype of the T cells is considerably different in in individuals suffering from chronic pain. Moreover there is a direct phenotypic link to the sex of the mammal but this connection is still currently under investigation [74]. Interleukin production and microglial cell stimulation are both character traits of the T-cells which increase the pain sensitization [75]. After the peripheral nerve injury, oligodendrocytes release interleukin-33. Interleukin 33 will act on the microglia and astrocytes [71]. Besides the normally effectuated myelinating function in the CNS the oligodendrocytes can also release IL-33 [76]. Cell damage triggers the release of IL-33 which has an molecular alarming effect and regulates the pathway of many cell types within the microenvironment among others the microglia and astrocytes [77]. A subtype of the glial cells are the astrocytes. Besides pH buffering by means of calcium and potassium, specialized functions such as signal transmission are also managed by astrocytes [78]. In the synaptic cleft, the astrocyte derived CCL2 function is, to increase monocytes and in particularly the T-lymphocytes. Studies in CCL2 deficient mice showed a much lower amount of monocytes in the CNS [79]. CXCL1 is a pro-inflammatory mediatory which has been demonstrated in macrophages, neutrophils and even epithelial cells. Furthermore astrocyte derived CXCL1's central function is it to increase the local monocyte infiltration [80].

6 “Substantia gelatinosa” and its role in nociception – possible ways to act on it

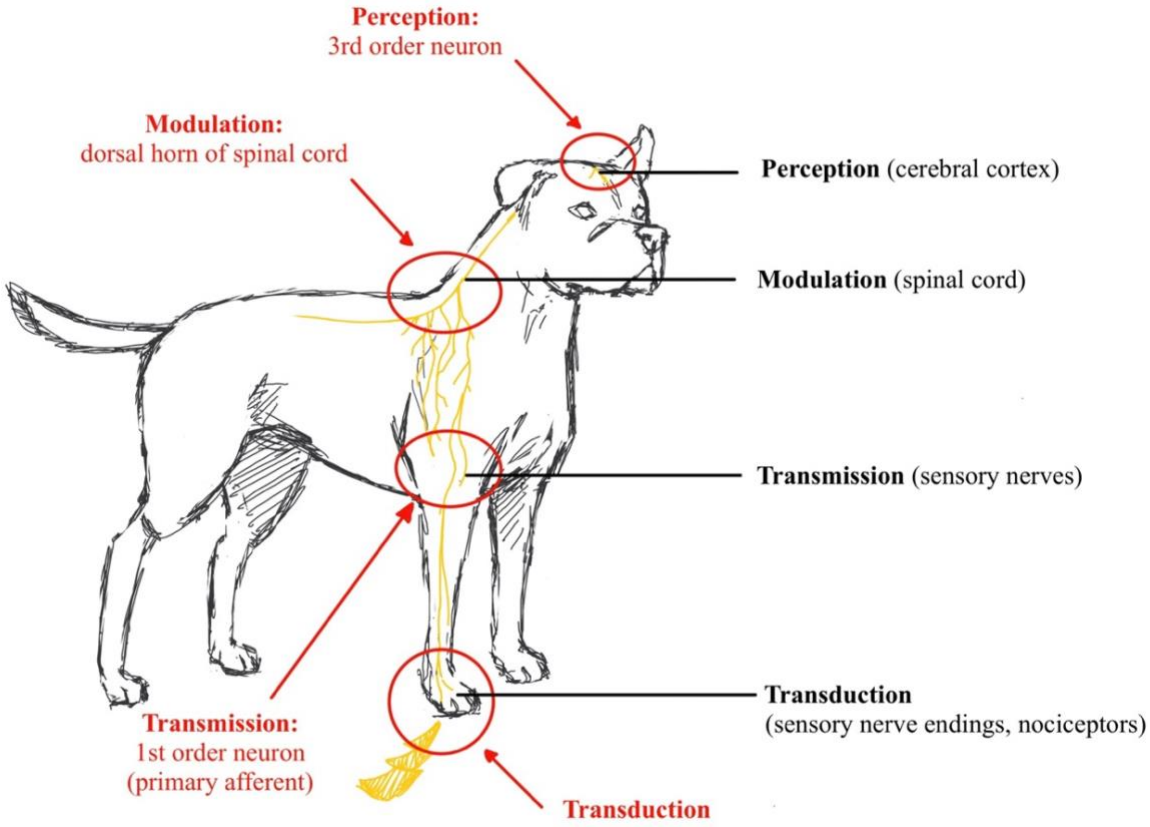
The substantia gelatinosa or “substantia gelatinosa of Rolando” (SGR) was first described by Luigi Rolando, an Italian anatomist. This neuroanatomical region in the spinal cord is characterized by the presence of neurons, unmyelinated axons and just a low number of myelinated fibers, neuroglial cells and dendrites. Within the spinal cord we can find the SGR as a thin longitudinal structure which runs dorso-laterally in the gray matter of the spinal cord. This is why we also sometimes call it the “gelatinous substance of the posterior horn of the spinal cord [81]. This spinal cord structure carries the name “gelatinous” in its name because of the high neuropil density. Neuropils are glial cells, unmyelinated axons and dendrites [82]. The SGR terminates in the medulla oblongata. It is in this location where it becomes the spinal trigeminal nucleus [83]. Within the spinal cord the SGR has a nociceptive modulatory and transmission function [84]. The modulatory function within the SGR is controlled by neurotransmitters. The main modulatory effect is influenced by the inhibitory transmitters GABA and glycine, the excitatory neurochemical glutamate and to a lesser extent by various other messenger chemicals such as serotonin, dopamine, biogenic amines and amino acids. While the morphological investigation of the neurons present in the SGR is ongoing nowadays we still cannot categorize them in the traditional sense but we rather group them based on their shape. The Spanish neuroscientist Cajal noticed interspecies differences of the neuronal cells found in the SGR after examining young dogs and cats. Subsequently he introduced the two main types by which these cells can be differentiated and termed them as “limiting cells” and “central cells”. Within the limiting cells there are “islet cells” which are GABAergic and within the central cells there are “radial cells” which can be GABAergic or glutamatergic. Thus the modulation in the SGR happens based on the modulation of the various firing frequency of the SGR neurons [81]. The inhibitory neurotransmitters GABA acts on the post-synaptic GABAa and GABAb receptors [85]. Typically, GABAa receptors are ligand-gated ion channels. They are used for quick synaptic inhibition which is mediated by chlorine influx. Decreased resting membrane potential leads to the inhibitory nociceptive effect. GABAb receptors however located pre-synaptically prevent intracellular Ca^{2+} influx by increasing the adenylyl cyclase activity. This results in a blockage of neurotransmitter release. As a result of these GABAergic actions the firing rate is reduced. The peripherally originating nociceptive stimuli passes through the SGR and

is dampened within this spinal cord structure before the stimuli information reaches the CNS [85].

Within the substantia gelatinosa alpha-delta and C-fibers are involved in transmission of nociceptive impulses. “Seoul National University Institutional Animal Care and Use Committee” demonstrated that the SGR contains 30-40% of inhibitory GABAergic interneurons which are known to play a key role in nociception modulation [86]. Pathological disease conditions can lead to a loss of these inhibitory interneurons. The resulting loss causes a greater incidence of central sensitization due to chronically high active state of the nervous system and a lower activational threshold [87]. Cats have an opioid sensitive superficial dorsal horn. [86] This GABA-mediated neurotransmission allows us veterinarians to interfere with the substantia gelatinosa’s ability to modulate nociceptive stimuli transmission [88]. Neuraxial opioid administration hinders the nociceptive synaptic transmitted stimuli to reach the CNS in its full intensity by inhibiting the liberation of excitatory presynaptic neurotransmitters such as substance P or glutamate. The reduction of excitatory neurotransmitters is directly linked to an overall local lower firing rate of the axons involved [81]. Despite many details of the presynaptic opioid pathway still being unknown and researched at this time, we can say that opioid induced GABAergic inhibition is mainly governed by presynaptic voltage-dependent potassium channels [88].

7 Modulation of pain and receptors that the drugs act on

The noxious stimuli are conducted along neuronal processes and can be modulated in 4 main points *(Figure 9)*. Modulation can happen at the 1) cortical level, 2) brainstem, 3) spinal cord and in the 4) body's periphery [90].



"Figure 9: A visual exemplification of the transduction, transmission, modulation and perception points in case of acute canine paw injury [89]".

1st approach - cortical level:

All somatosensory parts of the cortex represent cortical level modulation points such the prefrontal cortex (PFC), the ventrolateral cortex (VLO), the motor cortex (MC), the insular cortex (IC) as well as the anterior cingulate cortex (ACC). Two main mechanisms dominate the nociceptive modulation processes of these cortical areas. Firstly, there are direct corticospinal projections at the spinal cord which have a descending modulatory effect. Important brainstem structures like the periaqueductal gray (PAG), the locus coeruleus (LC), the rostroventral medulla (RVM) and the nucleus raphe magnus (RM) can be activated. Secondly the neuromodulatory substances and the

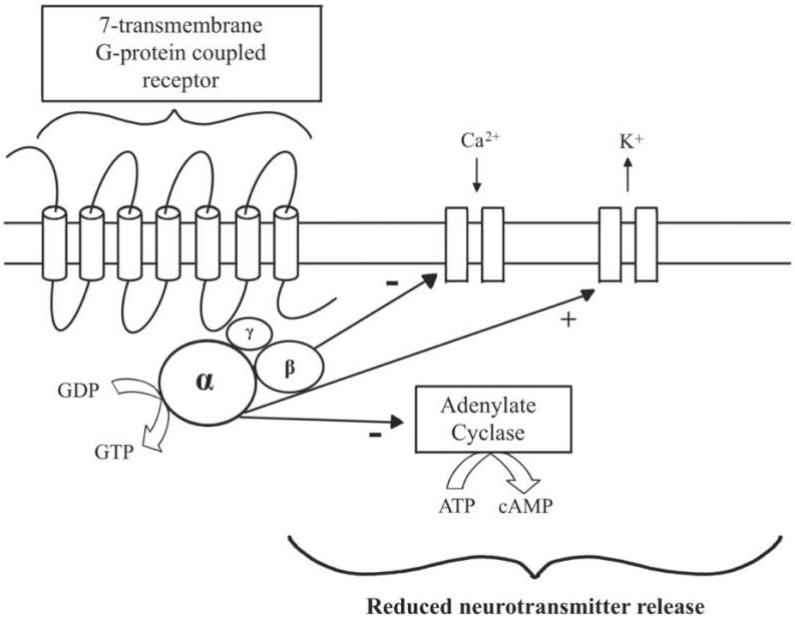
neurotransmitters of the cortical-subcortical and cortico-cortical areas can be used to modulate nociception. The cranially located PFC of the brain modulates acute as well as chronic nociceptive stimuli. The direct connection between the PCF basal activity and the nociceptive stimuli induced firing rate was observed. In chronic pain models the descending connection to the PAG was reduced. A reduced level of the excitatory neurotransmitter glutamate as well as a decreased gray matter density underline the PFC's ability to modulate the noxious stimuli [90]. Metabotropic glutamate group 1 receptors (mGLU1) are widely present in the mammalian brain. The role of these G-protein coupled receptors is to control the physiological transmission of glutamate and regulate the synaptic plasticity [91]. Nociceptive modulatory points in the PFC are altered by various other neurotransmitters. Dopamine originating from the ventral tegmental area's innervation and glutamate originating from the presynaptic alpha-1 adrenoceptors as well as the neurotransmitter noradrenaline can modulate nociceptive stimuli in the PFC [90].

The anterior cingulate cortex (ACC) is linked to nociceptive modulatory centers such as the PAG, the amygdala, the thalamic nuclei and the insular cortex and has projections running to the lumbar segments and dorsal horns of the spinal cord. Besides the nociceptive modulatory function, the ACC also has anxiety and emotional effects. Elevated excitability of the ACC is linked to the inhibition of hyperpolarization-activated cyclic nucleotide-gated channels (HCN1 channels) originating from the local upregulation of the mGLu1 [92]. Increased ACC function can be measures in mammals suffering chronic pain. Animal models demonstrated that the given hyperactivation can be shut off by triggering inhibitory neurons or via the application of various GABAergic substances. Modulation points are currently being discovered. In neuropathic pain, the D1 and D2 dopaminergic receptors are increased, thus drugs such as amantadine and dopamine reuptake inhibitors can act on these. Elevated numbers of M2 acetylcholine receptors can be found in neuropathic pain patients. The stimulation of these receptors decreased the nociception transmission. Interestingly taurine injection into the ACC is able to directly stimulate and activate the glycine type-A receptors which show a similar effect as the dopaminergic and opioid receptors [90]. Drug groups affecting the perception of the cerebral cortex include opioids, anesthetics, NSAIDs, benzodiazepines, phenothiazines and alpha-2 antagonists [89].

2nd approach – brainstem and spinal cord:

Opioid receptors can bind endogenous and exogenous opioids. Endogenous opioids are endomorphins, endorphins, enkephalins, dynorphins and orphanins. The clinically most often used exogenous opioids are fentanyl and morphine. The nociception orphanin-opioid receptor has only recently been described and is nowadays included as an opioid-like receptor. There are 5 types of opioid receptors which are subdivided within. There are mu-opioid receptors (MOR), delta opioid receptors (DOR), kappa opioid receptors (KOR), zeta opioid receptors (ZOR) and nociception opioid receptors (NOR). The MOR are subdivided into the mu-1, mu-2 and mu-3. DOR are subdivided into delta-1 and delta-2 and KOR into kappa-1, kappa-2 and kappa-3 subtypes [93].

Within the dorsal horn of the spinal cord, we can find the MOP pre-synaptically on the primary afferent neurons. As an effect of the stimulation of these receptors, the reduced release of glutamate decreased the nociceptive transmission capacity of the C and alpha-delta fibers. The receptors share a G-protein-coupled transmembrane anatomy. As the receptors get activated by intracellular transduction pathways GDP is transformed to GTP *(Figure 10)*. The beta-subunit of the receptors will decrease the Ca^{2+} influx and the alpha-subunit will increase the K^{+} efflux. In addition to this the decreased adenylate cyclase activity subsequently leads to a reduced neurotransmitter release. Morphine activation of the MOP receptors will in a similar way cause voltage sensitive calcium channels to close and induce a hypersensitivity by stimulating the potassium efflux. There will also be a blockage production of adenylyl cyclase activity leading to lower cyclic adenosine monophosphate. Overall, the reduced neuronal excitability will rapidly be induced by the blockage of neurotransmitter release. This will limit the transmission of nociceptive impulses and ultimately ends up in a lower pain sensation [94].



"Figure 10: A representation of a transmembrane opioid receptor leading to reduced neurotransmitter release [94]".

The cholinergic system in the spinal cord can also be considered as an additional modulation point. Application of cholinomimetics will induce analgesia by acting on the G protein-coupled muscarinic receptors and on the choline receptors. The anti-nociceptive property in the spinal cord comes from of cholinergic agent's ability to imitate the effect of released ACh. This results in blocking nociceptive information transmission which gives rise to a large therapeutic potential. The term "cholinomimetics" covers receptor agonists as well as agents that are able to block enzymatic activity. The stimulation of the inhibitory descending noradrenergic and serotonergic pain modulatory pathway triggers an increase of ACh in the spinal cord. The cholinergic interneurons of the dorsal horn release ACh [95]. The neurotransmitter acetylcholine acts on muscarinic-coupled G proteins, calcium, sodium and nicotinic ion channels [96]. Within the spinal cord's lamina II, the peripherally signal receiving neurons operate with glutamate. It has been demonstrated that the action of acetylcholine on these neurons will cause a reduction in the excitatory post-synaptic current which leads to reduced nociception transmission. The more ACh is present the less glutamate will be released which is considered as one of the main excitatory amino acids in the brain. ACh also acts on the increase of the post-synaptic inhibitory currents induced by the release of GABA. This second mode of action also acts very similarly

to the previous one as the higher the GABA baseline tone the lower the release of the excitatory glutamate. Rat studies show that drugs such as neostigmine or intrathecal ACh formulations are able to show a significant analgesic and antiallodynic effect [95].

Besides the ACh effect on glutamate and GABA, the analgesic effect can also be linked to glycine. This inhibitory neurotransmitter is linked to the activation of the spinal cord's muscarinic receptors. The STT's nociceptive information passes through the intralaminar parafascicular nucleus. This region also plays a role in the cholinergic analgesic system and reduces the nociceptive information transmission to the anterior cingulate cortex by means of ACh increase [95].

Neurokinin receptors are found throughout the CNS. The neurokinins peptides, substance P and opioids play an important nociceptive modulatory role in the nervous system. In the future the NK1 receptor antagonistic drugs may allow us to control the unwanted side effects of opioids such as respiratory depression and opioid analgesic tolerance. Currently there are many studies happening in this field [97]. Drug groups affecting the modulation at the spinal cord level include opioids, locoregional anesthetics such as lidocaine, NSAIDs, NMDA receptor antagonists, cholinesterase inhibitors, tricyclic antidepressants and alpha2 antagonists have been proposed as pharmaceuticals with modulatory effect [89].

3rd and 4th approach– transmission and transduction - sensory nerves and nerve endings:

Sensory nerves and free nerve endings are commonly clinically altered by the local, intravenous or peri-neural application of local anesthetics such as lidocaine.

The main target of local anesthetics are the sodium ion channels of the nerve cell membranes. Lidocaine's ability to diffuse freely through the neural sheets is changed once it reacted with H^+ ions in the axonal cytoplasm. Although alterations in pH such as in inflamed tissue are known to decrease the efficiency [98]. The analgesic mechanism is based on the alteration of the ion and ligand voltage gated channels as well as diverse G protein-coupled receptors. The potassium and sodium ion channels are blocked and then the ligand-gated Ca^{2+} channels are responsible for the regulation. The changes in the transmembrane potential have a direct effect on the resulting neuronal excitability. Metabotropic G-protein receptors are the commonly found

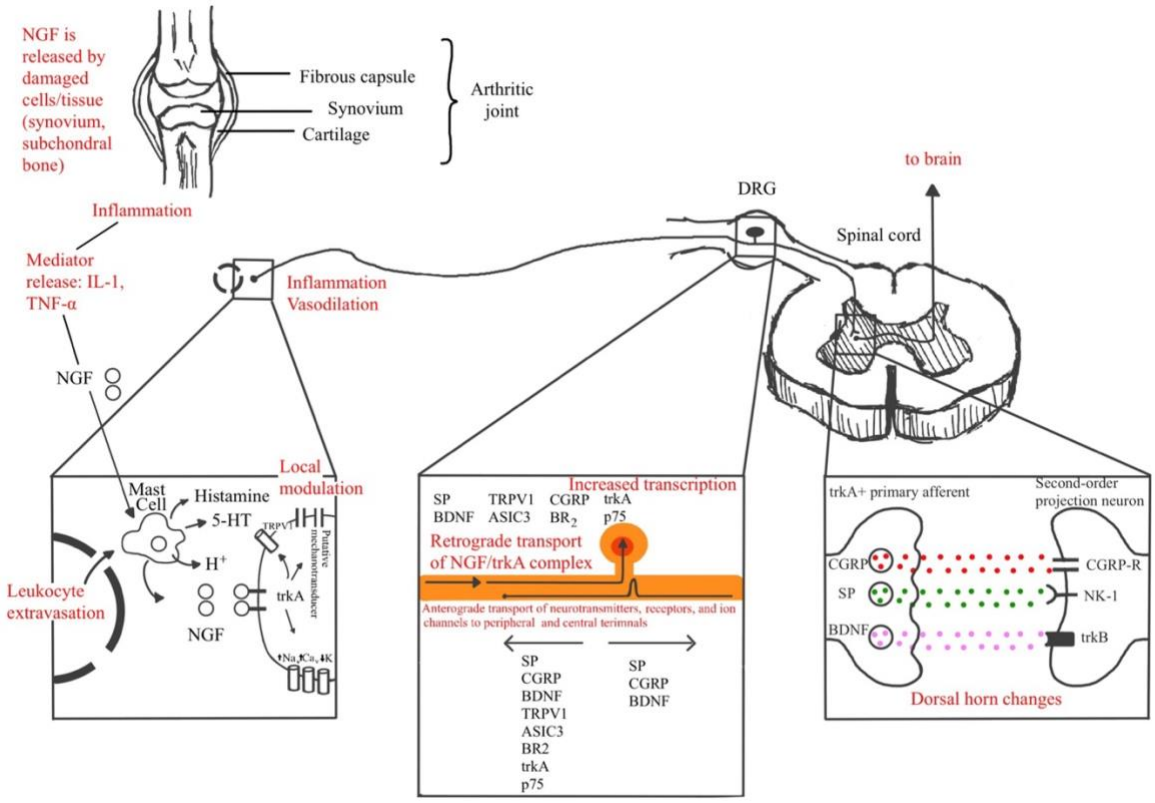
receptors and lidocaine inhibits the release of excitatory glutamate. Besides this, lidocaine also counters the effect of bradykinin which is known to be a really strong pro-inflammatory mediator and nociceptive substance. Bradykinin has its own specialized target receptors which are a type of G protein-coupled receptors. Here lidocaine binds the B2 receptors and hinders the neuronal activity leading to an analgesic effect [99]. In addition, the alpha-2 adrenergic receptor agonists receptors are divided into various subtypes. All subtypes cause hyperpolarization by limiting the adenosyl monophosphate and inhibition of adenylyl cyclase. As Ca^{2+} cannot enter the neurons anymore there will be strong reduction of neuronal firing [100]. Sensory nerve transmission can be altered with the application of local anesthetics and alpha-2 antagonists. Opioids, NSAIDs and local anesthetics are clinically applied to influence the transduction capacity of the sensory nerve endings [89].

8 Alternative ways to reduce pain

New nociception modulation possibilities and approaches arise to control pain, and these could very soon become the new norm. The management with new treatment methods is the future of nociception modulation and pain management in mammals.

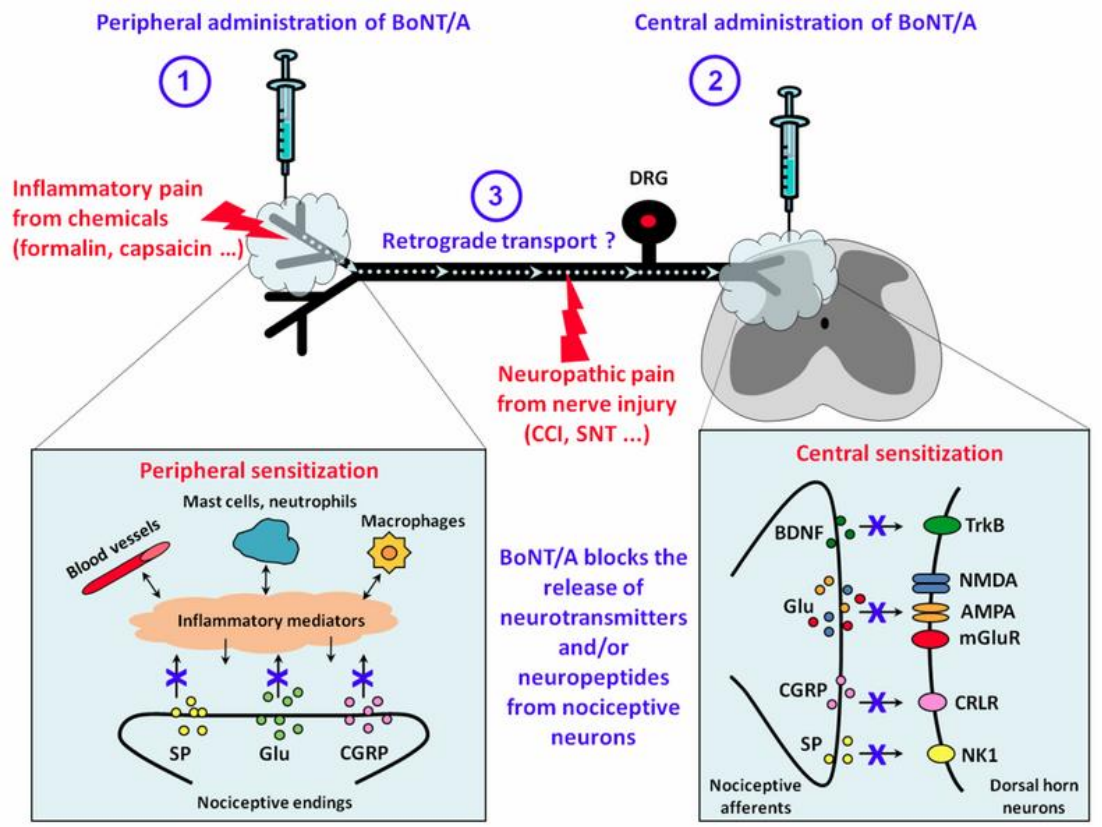
NGF is released by the inflammatory leukocytes such as macrophages, eosinophils and mast cells. As a result of the action of the proinflammatory mediators the nociceptors are sensitized and the feedback loop is maintained by the secretion of histamine, 5-HT, NGF and protons such as H+. The NGF will bind to the TrkA located on the unmyelinated C-fibers and the A-delta fibers terminals. There will be a complex formation. Retrograde transport is responsible for the complex movement to the DRG cell bodies. This will be the modulation point as the transcription of nociceptive substances and channels will be modified *(Figure 11)*. Voltage-gated sodium and calcium channels, transient receptor potential cation channel subfamily V member 1 (TRPV1), acid sensing ion channels, bradykinin receptors and putative mechanotransducers are all altered or affected. The TRPV1 channel plays an important role as the increased functioning of these channels will result in peripheral nociceptive hypersensitivity. The presence of the complex in the DRG also leads to the central sensitization by the surge of CGRP, SP and brain-derived neurotrophic factor (BDNF) [102]. BDNF can be located in many tissues and has an important role in the initiation of signal transduction pathways as it binds the TrkB and activates various signaling mechanisms [103]. The link of tropomyosin receptor kinase A (TrkA) to the NGF is the target of many currently running analgesia studies. The suppression of the NGF/TrkA signaling pathway poses a new treatment approach to chronically difficult to manage pain patients. Besides the demonstrated ability of NGF to generate perinatal sympathetic and sensory nerve growth we can also say that the immune and analgesic role of NGF/TrkA signaling is of significant importance [104].

Different drug mechanisms can be applied to alter the interaction of NGF with the tropomyosin kinase receptor. This complex ultimately triggers and maintains the nociception stimuli. There can be blockage of the TrkA function, or hindrance of the NGF/TrkA binding as well as sequestration of NGF. The most positive effects in clinical trials were demonstrated with the application of NGF sequestering antibodies. Due to the detrimental joint damage the clinical trials progress more slowly nowadays [105].



"Figure 11: The NGF's mechanism in relationship to the nociceptive system responsible for the prolongation of the pain sensation [101]".

Mesaconitine has been proven to provide muscular analgesic effect by acting on the noradrenergic system and on the serotonin system. The effects can be compared to the TCA and NA reuptake-inhibitors, but the side effects are much lower. TCA drugs have the tendency to be cardiotoxic and commonly cause atropine like side effects which in return, clinically speaking, limits their use mammalian pain management protocols [106].



"Figure 12: Novel botulinum approach and its effects of both the central and the peripheral sensitization cascade [107]".

A recently proposed solution to this type of nociceptive stimuli leading to long term sensation of pain is the peripherally or centrally targeted application of botulinum toxin. The application of the purified botulinum toxin serotype A against myofascial pain syndrome and trigeminal neuralgia is undergoing clinical trials. These trials solely include clostridial toxins limited to the nervous system. The botulinum neurotoxins (BoNTs) synergistically interact with either synaptic vesicle proteins 2, synaptogamin or gangliosides which are the pre-synaptically located on the neuronal membrane *(Figure 12)*. In the neuronal cytosol the vesicles will trigger synaptobrevin, sintaxin and synaptosomal associated protein of 25kD (SNAP-25) release. Cholinergic as well as non-cholinergic nerve terminals are the main target of BoNTs. The nociceptive stimuli modulation capacity comes from the neuropeptide and neurotransmitter stimulation capacity. Certain substances such as CGRP and SP may be completely inhibited in their release while other substances such as GABA, noradrenaline, aspartate, glutamate and catecholamines, monoamine and dopamine remain in the chromaffin cells, central neurons or cerebral synaptosomes. Hindrance of neuropeptide

release of SP or CGRP is considered as a possibility to treat patients suffering from neuralgia [107]. In thedorsal horn the CGRP will act on the CGRP- receptors of the second order projection neuron, SP on the neurokinin 1 receptor and BDNF will act on the TrkB [101]. Animal models of NGF–sequestering agents show that the analgesic effect can be ensured even in case of colonic hypersensitivity or continuous degradation of the bones and joints. It is accepted that the specificity of the antibodies limits the inflammation induced mediators. Despite the benefits, the entire class anti-NGF often show treatment related side effects such as extremity pain and peripheral edema. The entire tropomyosin family can be blocked using the TrkA inhibitors [101].

In human medicine PTSD can be treated with MRI guided laser therapy targeting the right side of the amygdala. This is a regularly applied technique proposed reoccurring epilepsy patients. This approach is not yet done in veterinary medicine, but this might become a novel approach in the coming years. In our clinics, difficult to manage, or pharmaceutical treatment-resistant pain patients might benefit from this surgical intervention in the near future [108].

Cryotherapy means the freezing or severe cooling of individual nerves and or a group of nerves in a given region of the body. Despite many aspects still remaining unclear we can say that cryotherapy has an effect on the nerve terminals. The analgesic effect of the nerve terminals is linked to the reduced transduction speed of the nerve fibers. This in return will increase the tolerance and threshold towards the nociceptive stimuli [109]. An example of precisely applied cryotherapy is the local application in the ventrolateral quadrant and the dorsolateral funiculus of the spinal cord. It has been demonstrated that surgical lesions and cold probes, if applied in the exact location of the spinal cord, will reduce the nociceptive information transmission, thus resulting in an analgesic effect [110].

Acupuncture induced analgesia has a long therapeutic history and a wide band of therapeutic analgesic applications. The full analgesic mechanism details are still being researched. The cutaneous-receptor and muscle-spindle-rich points of the body are believed to be the most efficient sites for acupuncture, but the scientific underlying

anatomy remains unclear these days. Precise acupoint stimulation is done with small metal needles with or without additional electrical stimulation. The cascade starts with the activation of local receptors which ultimately will send the stimuli to the brain. One hypothesis is that the keratinocytes and fibroblasts of the acupunctured tissue will release ATP which is subsequently rapidly broken down. The analgesic effect comes from the released adenosine which binds to adenosine A1 type receptors. After acupuncture mast cells which have TRPV2 channels, and are thus sensitive to mechanical, and thermal irritation, will degranulate. Afferent fibers are stimulated by the resulting mild local tissue irritation and the effect of the proinflammatory mediators [111]. The effect of endorphins on the neuronal pathway are widely considered as an approved acupuncture mechanism theory [112]. After the impulse has travelled to the spinal cord dorsal horn the message will be passed on to the PAG, NRM and to the arcuate nucleus. The released monoamines such as norepinephrine and serotonin will act at the level of the spinal cord and the endorphin and enkephalin neuropeptides act on the spinal level and in the brain. The neurons of the periaqueductal gray store and respond to released endorphins and will induce analgesia [113]. The PAG released endorphins will also act on the nucleus raphe magnus induced opioid driven analgesia [114]. Interestingly the effect of the opioidergic neuropeptides cannot be reverted with naloxone. Additionally, the analgesic effect is underlined by the hypothalamic-pituitary adrenal axis which will result in a decrease of cyclooxygenase-2 and prostaglandin E2. Peripheral dopamine and opioid peptides also aid in pain inhibition by lowering the inflammation [111]. A desensitization of the peripherally located nociceptors happens because of the released opioids, the increased secretion of GABA as well as the decreased presence of substance P and of various other excitatory amino acids. The reduction of the pro-inflammatory cytokines is directly linked to the alleviation of inflammatory pain. Spinally acting norepinephrine and serotonin are responsible for a lower phosphorylation of the n-methyl-d-aspartate (NMDA) receptors and as a result of this reduced phosphorylation there is a lower level of excitability in the spinal cord [115]. Serotonin does this by binding to 5-HT1A receptors. Norepinephrine derived from the synaptic locus coeruleus has an analgesic effect by binding to the alpha2-adrenoreceptors of the primary afferent nerves in the spinal cord. The nociceptive suppressing effect comes from the resulting inflammatory mediator induced beta-endorphin release [116].

Surgical pioneers very early on also consider the DRG as being an interesting target to modulate and alleviate peripherally originating nociceptive stimuli. Interventions such as dorsal root rhizotomy meaning the transection of nerve fibers by means of electrical current application, chemical burning, pulsating radiofrequency application, dorsal root entry zone lesioning and gangliectomy all have their well-recognized position in neuromodulation therapy [10]. In the future veterinary intractable pain patients may also benefit analgesia from deep brain stimulation (DBS) techniques [117]. This neurostimulation surgery implants electrodes into the thalamus and subthalamic regions. Currently this approach is used in human medicine for large variety of pain disorders but this could soon become a novel approach for our really high valuable clinical patients [118].

9 Discussion

The objective of this literature review is to gain a deeper understanding on the physiological function of mammalian nociceptive pathways and to get a good comprehensive overview of the main nociceptive points which we, as practicing veterinarians or surgeons, can influence. This paper includes many articles and studies which were published in the previous 5 years. The focus was put to utilize articles of veterinary origin directly related to animal studies. Some of the included articles about the "ascending and descending pathways" as well as some papers about the "substantia gelatinosa of Rolando" date further back. These pathways and structures of the nociceptive system were described years ago. This being said, additional studies with recently discovered details were also included. As pain research is much more promoted in human medicine these days, some in depth studies about novel pain therapy approaches, which are universally applicable in the mammalian class, originated from the human medicine publications.

To be able to appreciate the full potential effect of the nowadays available opioids we shall take the cholinergic system into account. The increase of inhibitory transmitters and a reduction in excitatory transmitters will allow the enhancement of the opioid's effect. This interaction has recently been proven in rat tail flicking tests. Neostigmine in combination with opioids can be considered a "synergism" as this will underline the morphine's wanted effects. This knowledge must be applied carefully as an overdose in acetylcholinesterase inhibitors will lead to an overstimulation of the nicotinic and muscarinic receptors ultimately leading to the disagreeable death of the patient due to respiratory failure [95]. Damaged nerve cells produce and release leukocytes and T cells. These produce and release opioid peptides. While 50% of the opioid peptide containing immune cells are T cells, the T cells only contribute to 11% of the nerve cells' infiltrated leukocyte. This novel information opens a large potential to new therapy approaches in the future. The combined application of functionally modified T cells originating from genetically immune-modified animals could soon become a supplementary approach to treat difficult inflammatory and neuropathic nociceptive cases [74].

The current medicine is seeing great improvements both in research and development of new therapy approaches to solve the most diverse causes of disease and pain. Mammalian nociception is often the result of pathological diseases or an external of internal physical imbalance leading to inflammation and decreased life quality. The scientific papers included in this thesis were evaluated based on the qualitative information included in the studies, the

species involved, and the real informative value added. Many detailed aspects of the nociceptive pathways are still not fully clear or and not understood. The identification of new receptors, neurotransmitter and up and down-regulatory pathways are enriched with new details day by day. The potential faults and risks associated with this ongoing research in the hunt for details is that many papers include “currently accepted theories”. These are therefore directly bound to many limitations such as the full understanding of the mechanisms involved in phantom pain after a limb amputation, the causes of neuralgia development and the diagnosis thereof in the veterinary clinic, the feedforward and feedback loops of the acupuncture in relationship to the spinal cord and brain, the exact functioning of the presynaptic opioid pathway and the exact location and action of the TRPM8 receptors. Once the nociceptive impulse reaches the mammalian cortex the information enters the cortical columns. This miniature structure is of high relevance regarding the full understanding of the nociceptive pathway in connection to the psychological aspect of pain. This is also still a medical gap which will hopefully be explained sometime in the near future. Further interesting research fields include the modulatory function of the periaqueductal gray and of the locus coeruleus.

10 Summary

In ancient times nociception and pain were considered a philosophical question rather than a scientific phenomenon [120]. But we can appreciate many aspects of the nociceptive pathway despite minor details of certain cascades still being under investigation nowadays. With stronger knowledge on the functional aspects and on the substances involved in the nociceptive cascade we will be able to provide a more complete spectrum of pharmaceutical and / or surgically based analgesic approaches to our patients whilst aiming for the lowest possible amount of acceptable side effects.

The physiological transduction and transmission of nociceptive stimuli from the periphery to the CNS is based on a neuroaxis composed of an ascending and a descending pathway. Within the grey matter of the spinal cord, the more important spinothalamic tract (STT) of the ascending pathway is involved in the transmission of nociceptive stimuli from the periphery to the brain. The descending pathway is connected to the motor pathway for extremity withdrawal in case a noxious stimulus acts on the mammalian tissue. In addition, the stress induced analgesia by means of endogenous opioid and cannabinoid receptors also plays part of the descending pathway. In the STT, the peripherally originating nociceptive stimuli are passed from the first to the second and finally to the third order neuron. The third order neuron then passed through the thalamus and ends up in the cortical columns of the somatosensory cortex. This is the location where the nociceptive stimuli are transformed to a sensation of pain.

Diverse types of nociceptive stimuli can lead to a broad band of different pain types. While functional pain originates from a non-visualizable source, the inflammatory pain uses histamine, pro-inflammatory mediators and substance P to increase the transmission of the peripherally sensitized free nerve endings to the CNS, ultimately leading to a stronger pain sensation. Neurogenic pain patients are suffering from a chronic central or peripheral nervous system dysfunction leading to constant hyperalgesia.

Not all mammalian nerve fibers are the same and we differentiate them based on the presence or absence of myelin sheaths, diameter, conduction speed and function. While the myelinated Aδ are responsible for the fast pain transmission which is well localizable, the unmyelinated C-fibers are involved in slow pain transmission. Besides this the interneurons are able to either increase or decrease the frequency of the CNS targeted nociceptive stimuli.

After peripheral tissue injury, the free nerve endings start a nociception cascade. The nociceptor will encode the noxious stimuli in form of an action potential which subsequently runs along the first order neuron to the spinal cord. For this action potential to form, the activational threshold needs to be reached. Many pro-inflammatory mediators decrease the activational threshold of the nociceptors. The resulting increase in firing rate frequency causes a stronger nociceptive signal to be sent to the CNS. Diverse channels located on the free nerve ending induce a depolarization of the first order neurons which leads to the induction of this action potential. Besides the surrounding tissue having an impact on the free nerve ending's ability to accurately capture the nociceptive information, the spinally located dorsal horn and the DRG can initiate the "central sensitization" by lowering the needed threshold. This happens by briefly lowering the activation threshold by means of phosphorylation of various ion channels. Generally speaking, in their resting state the nociceptors are not sending any action potentials and remain in an "inactive state" but once the threshold is reached, they react with an all-or none type of response. Dorso-laterally in the spinal cord there is the substantia gelatinosa of Rolondo which runs cranially into the medulla oblongata and which plays an important modulatory function in the spinal cord. Inhibitory neurotransmitters such as glycine and GABA and to a lesser extent excitatory neurotransmitters such as dopamine and serotonin are locally responsible for this modulation. Alteration of the firing frequency is the main way the substantia gelatinosa of Rolando is modulated. Here the postsynaptic GABAa receptors serve for quick inhibition as they can act on the on ligand-gated chlorine influx and the pre-synaptic GABAb receptors block the intracellular Ca^{2+} influx. As a result, there is reduced neurotransmitter release. Neuraxial administration of opioids inhibits the excitatory neurotransmitter release thus the nociceptive stimuli reaching the CNS is dampened.

The mammalian nociceptive pathway is subdivided into transduction which happens at the sensory nerve endings, transmission along the nerves, modulation in the spinal cord and the perception in the cerebral cortex. The emotional aspect of nociception and the memory thereof is also a part of the mammalian sensation of pain and this emotional aspect is managed by the brain's anterior cingulate cortex. The entire somatosensory cortex and the associated neurotransmitters are involved in mammalian nociception modulation. Endogenous and exogenous opioid receptors play an important analgesic role in the brainstem and in the spinal cord. As a result of receptor activation there is a reduced excitatory neurotransmitter release as well as a reduced Ca^{2+} influx but an increased K^{+}

efflux. This leads to lowered neuronal excitability. The application of cholinomimetics allows the blockage of enzymatic activity which spinally speaking results in increased ACh levels. Considerably lower levels of excitatory neurotransmitters are present if there is a slight increase in ACh. This explains the inversely linked analgesic effect. Sensory nerve endings are clinically often altered by application of local anesthetics which can diffuse through the neural sheath to alter the ion and voltage gated channels. As a result, K^+ and Na^+ channels are blocked causing a change in the transmembrane potential which will reduce neuronal excitability. Pro-inflammatory mediators such as bradykinin, which also can be regarded as pro-nociceptive mediators, can be countered via the application of local anesthetics such as lidocaine. Alpha-2 adrenergic receptor agonists induce hyperpolarization after binding their receptors which blocks the Ca^{2+} from entering the neurons leading to a strong reduction in firing rate and thus the patient will sense analgesia.

Despite the classical pharmacological approaches there are many more conservative alternative approaches to reduce mammalian pain. In the recent years a lot of focus has been put onto the NGF's ability to modulate nociceptive stimuli. The leukocytes will release proinflammatory mediators which sensitize the nociceptors. Once the NGF gets into contact with TrkA there is a complex formation which undergoes retrograde transport to the DRG where the transcription of nociceptive substances is increased. Inhibiting the NGF/TrkA binding is directly linked to a reduction in nociceptive stimuli reaching the CNS. Clinically the most promising results were demonstrated with NGF sequestering antibodies despite the severe joint damaging side effects. Peripherally applied botulinum toxins target the cholinergic and the non-cholinergic nerve-terminals in a way that there is a release of synaptobrevin, sintaxin and synaptosomal associated protein within the neuronal cytosol which is directly linked to the neurotransmitter release. The release of substance P and CGRP may be completely inhibited leading to a diminished sensation of pain. Despite the cryotherapy and acupuncture induced analgesia being known for a really long time, many detailed aspects still remain unclear nowadays and are undergoing research. We can certainly say that the application of low temperature is directly linked to the reduced neural transduction speed. The lower transduction speeds leads to the analgesic effect. A widely accepted hypothesis regarding acupuncture is that it is based on the rapid breakdown of ATP. The released adenosine will then interact with the mast cells and cause them to degranulate. Within the neuronal pathway endorphins play an important role in the spinal cord and brain and norepinephrine as well as serotonin act spinally. The activation of the HPA axis lowers

the prostaglandin E2 and cyclooxygenase-2. Additionally, there is a peripheral opioid increase, and a reduction of the pro-inflammatory mediators. The combination thereof is known to alleviate inflammatory pain.

Surgical approaches targeting the DRG such as dorsal root rhizotomy are a more direct approach to interacting with the nociceptive pathway. Despite the important role of the surgical interventions in neuromodulative therapies, there are always limitations to direct surgical nerve insult such as phantom pain. In the future we might be able to clinically manage such a pain disorder much more easily but with our current worldwide medical knowledge, such patients still challenge veterinarians on a daily basis.

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I would like to end this thesis paper with these 2 quotes:

“You don’t want your neurosurgeon to have doubts about the meaning of it all while he or she is operating on your brain.” – by Aleksander Hemon

“A candle loses nothing by lighting another candle.” – by James Keller

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Thesis progress report for veterinary students

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Thesis title: The physiology and modulation of nociception in mammals

A fájdalomérzékelés élettana és szabályozása emlősökben

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	December	4th	Presentation of various topics + choosing of topic	
2.	2024	March	2nd	Presentation of various papers, documents and sources to prepare the thesis writing	
3.	2024	March	26th	Presentation of the current version + citation management	
4.	2024	June	14th	Legal rights of usage and author contact was discussed	
5.	2024	June	15th	Verification if the thesis was still in line with the original topic title	

Grade achieved at the end of the first semester: jeles (5.)

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2024	August	1st	Formatting adjustment	
2.	2024	August	5th	Management of figure citations	
3.	2024	August	23rd	Adjustment of margin size + layout	
4.	2024	August	24th	Plagiarism checker was discussed	

5.	2024	August	31st	Typing errors were removed	

Grade achieved at the end of the second semester: jeles (5)

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

.....
signature of the supervisor

Signature of the student: .....

Signature of the secretary of the department: .....

Date of handing the thesis in: 2024 october 31.