ACUPUNCTURE AS TREATMENT FOR DOGS SUFFERING FROM CHRONIC PAIN

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Tiivistelmä - Referat – Abstract
Pain is an unpleasant feeling bound to affect us, both humans as animals, during our lifetimes. Thousands of people are suffering from chronic pain around the world, and chronic pain in animals and ways to treat it is rapidly gaining more and more interest. The pain network is a vastly intricate one, with complex interactions between a plethora of neurons and cells. Modern science has yet to shine a light on the complete process of pain sensation. Acupuncture has been used for thousands of years in treating pain amongst other problems and is today approved by the World Health Organization as a treatment for certain types of pain among other conditions. Wide research has been carried out during the last few decades as acupuncture is gaining ground in the Western world and while evidence of its analgesic effects and some mechanisms of action (e.g. endogenous opioid-release) have been found through studies, our understanding of the response elicited by acupuncture still remains incomplete. In the current study, material was gathered in form of questionnaires, which owners to dogs treated with acupuncture filled out. We then assessed the efficacy of acupuncture as a treatment method for dogs suffering from chronic pain by analysing improvements in mobility, quality of life and pain by means of the Helsinki Chronic Pain Index (HCPI), visual analogue scales (VAS) (n=5-9) and a comparative enquiry (n=85). Although no statistically significant differences were found, results were constantly indicative of improvement, and significant differences might have been found were it not for the small numbers of cases in the HCPI- and VAS-studies. While no conclusions can be drawn from the current study, the results may be guardedly interpreted as indicative of the analgesic abilities of acupuncture in treating chronic pain in dogs.

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Tiivistelmä - Referat – Abstract

Smärta är en obehaglig känsla som var och en av oss, såväl djur som människor, upplever under sin livstid. Tusentals människor världen över lider av kronisk smärta medan kronisk smärta hos djur samt behandlingsmetoder därav är ämnen av snabbt växande intresse. Den del av nervsystemet som förmedlar smärta är ett intrikat nätverk bestående av komplexa interaktioner neuroner och celler emellan. Processen som leder till smärtupplevelsen är i den moderna vetenskapens ögon ej ännu komplett klarlagd. Akupunktur har använts som behandling för smärta och andra åkommor i tusentals år och är idag godkänt av Världshälsoorganisationen WHO som behandlingsmetod för bland annat vissa typer av smärta. Efterhand som akupunktur under de senaste årtiondena vunnit mark i västvärlden har vidsträckt forskning utförts i ämnet. Trots att bevis för akupunkturens smärtlindrande effekt påvisats samt vissa verkningsmekanismer, såsom frisättande av endogena opioider, klargjorts, är vår förståelse för kroppens respons på akupunktur än så länge ofullständig. Den aktuella forskningsstudien är baserad på material från insamlade blanketter, som ägare till hundar som behandlades med akupunktur fyllde i. Vi bedömde akupunkturens effektivitet som behandling för hundar som lider av kronisk smärta genom att analysera förbättring i rörlighet, livskvalitet och smärta. Dessa indikatorer mättes med hjälp av Helsinki Chronic Pain Index (HCPI), visuella analoga skalor (VAS) (n=5-9) samt en jämförande enkät (n=85). Även om inga statistiskt signifikanta skillnader påträffades, var resultaten genomgående indikativa för förbättring och signifikanta skillnader kunde eventuellt ha uppdagats om det inte vore för det låga antalet case i de HCPI- och VAS-baserade studierna. Trots att inga slutsatser kan dras på basis av den aktuella studien, kan resultaten varsamt tolkas som indikativa för akupunkturens positiva verkan som behandlingsform vid kronisk smärta hos hundar.

Avainsanat - Nyckelord - Keywords
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Summary

Pain is an unpleasant feeling bound to affect us, both humans as animals, during our lifetimes. Thousands of people are suffering from chronic pain around the world, and chronic pain in animals and ways to treat it is rapidly gaining more and more interest. The pain network is a vastly intricate one, with complex interactions between a plethora of neurons and cells. Modern science has yet to shine a light on the complete process of pain sensation. Acupuncture has been used for thousands of years in treating pain amongst other problems and is today approved by the World Health Organization as a treatment for certain types of pain among other conditions. Wide research has been carried out during the last few decades as acupuncture is gaining ground in the Western world and while evidence of its analgesic effects and some mechanisms of action (e.g. endogenous opioid-release) have been found through studies, our understanding of the response elicited by acupuncture still remains incomplete. In the current study, material was gathered in form of questionnaires, which owners to dogs treated with acupuncture filled out. We then assessed the efficacy of acupuncture as a treatment method for dogs suffering from chronic pain by analysing improvements in mobility, quality of life and pain by means of the Helsinki Chronic Pain Index (HCPI), visual analogue scales (VAS) (n=5-9) and a comparative enquiry (n=85). Although no statistically significant differences were found, results were constantly indicative of improvement, and significant differences might have been found were it not for the small numbers of cases in the HCPI- and VAS-studies. While no conclusions can be drawn from the current study, the results may be guardedly interpreted as indicative of the analgesic abilities of acupuncture in treating chronic pain in dogs.

Content

Introduction

Both humans and animals suffer from chronic pain, often resulting in attenuation of the use or direct misuse of the locomotor apparatus, i.e. altered movement-patterns because of pain. Treatment of this type of chronic pain is seldom simple, with a wide array of treatment-methods to choose from, some more efficient than others. A highly debated pain treatment method is acupuncture, knowledge and use of which has expanded widely in the Western hemisphere during recent decades. The aim of this licentiate thesis is to present some of what research has found regarding pain, acupuncture and acupuncture analgesia and assess the use and perhaps efficacy of acupuncture in treatment of dogs with chronic pain, the hypothesis being that acupuncture does alleviate chronic pain.

1 PAIN

Pain, defined by the International Association for the Study of Pain (International Association for the Study of Pain 2012) as ”an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”, is something that those capable of experiencing - humans as well as animals - are extremely likely to encounter during their lifetimes. Pain is an important symptom of many diseases, its function being to prevent (further) tissue damage and promote the healing of injured tissue (Raouf et al. 2010). The perception of pain is highly subjective (Beecher 1952) and therefore difficult to measure. Moreover, the pain experience is thought to consist of three dimensions; a sensory-discriminative, a motivational-affective and a cognitive-evaluative dimension (Melzack and Casey 1968), which makes the pain sensation that much more complex to study and to understand. This complexity is reflected in, for example, the thousands of people around the world suffering from poorly manageable chronic pain (Breivik et al. 2006, Johannes et al. 2010). While the prevalence of chronic pain in dogs is unknown, the ability to recognise and assess it is growing and the importance of treating it is rapidly becoming clearer to both owners and veterinarians.

1.1 Classification of pain

Pain can be categorized in a variety of ways, and it seems the classification of pain continuously changes parallel to the growing knowledge about pain. One way of broadly categorizing pain is into the three groups of nociceptive, inflammatory and pathological pain (Woolf 2010). Nociceptive pain is the sensation which stems from the body's detection of a noxious stimulus, i.e. a warning signal of potential tissue damage. The sensation results in a withdrawal reflex, with the aim to protect the body from further injury. Inflammatory pain rises from the immune system's response to tissue damage or infection. Inflammation hypersensitizes the injured area making it extra painful, thus aiding in the healing process by protecting it from further stimulus or damage (Woolf 2010).

In contrast to nociceptive and inflammatory pain, which serve the purpose of protecting the body, pathological pain is rather a state where the nociceptive signal processing in the nervous system has maladapted such that the pain threshold is lowered and the nociceptive signals are amplified in the central nervous system (CNS) (Woolf 2010). This can occur in case of nerve injury (neuropathic pain) and in some diseases where no damage or inflammation is present (dysfunctional pain, e.g. fibromyalgia, irritable bowel syndrome) (Woolf 2010). Cancer pain seems to be a unique type of (pathological) pain (Honore et al. 2000, Schmidt et al. 2010).

1.2 Acute and chronic pain

The division of pain into acute and chronic is not as easy as it seems. Chronic pain has been classified as pain that extends past the normal expected time of healing (Bonica 1953), with normal healing times defined as e.g. one, three or six months, depending on the disease process in question (International Association for the Study of Pain 1994). Some diseases, however, continue to generate pain even though healing has never occured (e.g. osteoarthritis), or heal first after which it may recur (e.g. migraines).

1.3 The pain pathway

The organism's pain-receptive, -referring and -translating pathway, or network if you will, is a vastly intricate one. The next chapter will focus on this pain pathway, going through its neuroanatomy and furthermore the biochemistry embedded in it as it looks in the light of science today. As we will see, the path of the noxious stimulus goes from nociceptor to the spinal cord to the brain, where it is processed. The brain then sends signals for the body to react (e.g. increase in heart rate) as well as modulates the pain (e.g. release of analgesic components) by sending descending signals through basically the same pathway from whence the stimulus came.

1.3.1 Nociception at peripheral terminals

Noxious stimulus is detected and encoded by specialized peripheral sensory neurons called nociceptive neurons or nociceptors (Sherrington 1906). Nociceptors, which are primary afferent neurons, are found all throughout the body; in the skin (Sherrington 1906), muscle (Mense and Schmidt 1977), joints (Burgess and Clark 1969) and the viscera (Ness and Gebhart 1990). The most distal part of the nociceptors that detects the noxious stimulus, the receptive terminal, consists of free nerve endings branched tree-like from the axon. The endings end in an end bulb, and some endings possess additional axonal expansions that contain different types of messenger molecules. The nociceptive neurons often contain neuropeptides, such as substance P (SP) or calcitonin gene-related peptide (CGRP) (Mense 2008).

Nociceptors are generally silent, and evoke action potentials only when stimulated sufficiently (Sherrington 1906). Nociceptors are activated by high-threshold stimuli as opposed to ”normal” sensory receptors, that are very sensitive to stimuli and activate from low-threshold stimuli (Bessou and Perl 1969). Nociceptors also generally react to more than one modality of stimulus, e.g. heat, mechanical and chemical stimuli, and are therefore also called polymodal receptors (Bessou and Perl 1969, Davis et al. 1993). The more research has been carried out on nociceptors, the more it has become clear that nociceptors are a vastly heterogenous group, that by their action probably have a larger role in the nuances of pain (e.g. aching, pricking, throbbing, burning) than we realize.

Nociceptors can be subdivided in several manners according to characteristics such as conduction velocity, form of stimulus that evokes a response (e.g. heat, mechanical), response characteristics and distinct chemical markers (e.g. membrane receptors or peptides they are releasing) (McMahon et al. 2013). Grouping nociceptive afferents by conduction velocity, there are two main groups; the fast conducting, myelinated A- and the slower conducting, unmyelinated C-fibre afferents (conduction velocities >2 m/s and <2 m/s respectively). The C-fibre afferents are believed to conduct a burning pain sensation, whereas the A-fibre afferents are believed to evoke pricking or aching pain in addition to the feeling of sharpness (McMahon et al. 2013). Nociceptors can further be divided into mechanically sensitive afferents (MSAs) and mechanically insensitive afferents (MIAs) based on their ability to detect (noxious) mechanical stimuli (Meyer et al. 1991). Most nociceptors belong to the MSAs, but some belong to the MIAs, meaning that they have a very high threshold or no sensitivity at all for mechanical stimuli. They can, however, detect other stimuli (Meyer et al. 1991).

Nociceptors are also divided into peptidergic and non-peptidergic, based on binding to isolectin B4 (IB4) (Silverman and Kruger 1988b). Non-peptidergic neurons contain fluoride-resistant acid phosphatase (FRAP) (Silverman and Kruger 1988a) and bind to IB4 (Silverman and Kruger 1988b), whereas peptidergic neurons don´t bind to IB4 and contain SP and CGRP among other peptides. Considerable coincidence between positive IB4-binding and/or FRAP- and SP- & CGRP-containment in nociceptors has been found in rats though, but less so in mice (Carr et al. 1990, Wang et al. 1994, Bergman et al. 1999). The signal transducing receptors expressed on the peripheral terminals of nociceptors differ between peptidergic and non-peptidergic neurons, which partly might explain why the sensitivity to a given stimulus differs between nociceptors (Vulchanova et al. 1998, Zwick 2002). Species differences in receptor-expression and co-existence among various markers also is apparent (Zylka et al. 2003).

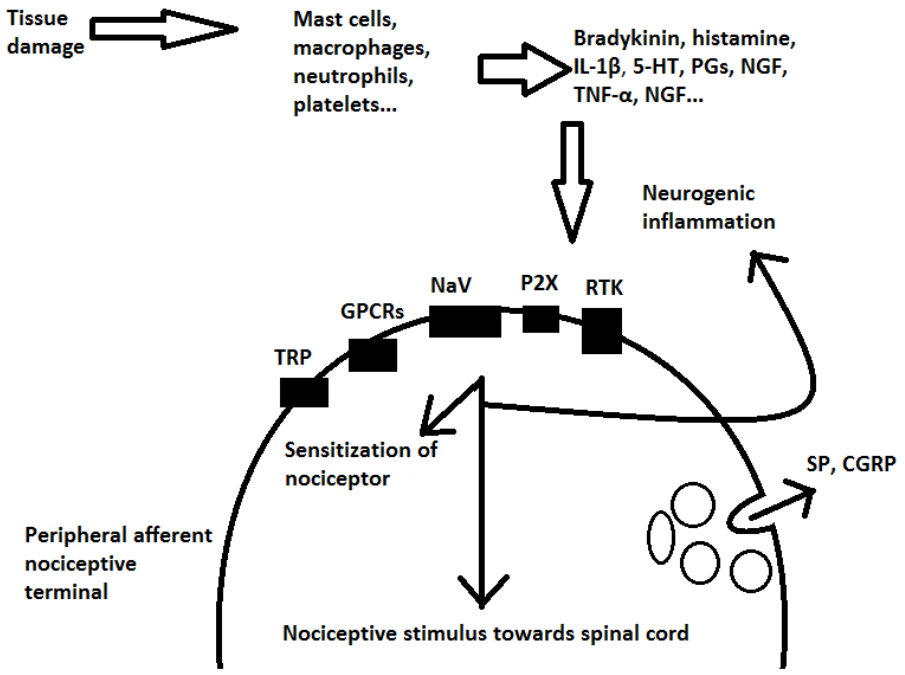

Figure 1. Nociception at the peripheral terminal. Tissue damage leads to activation of e.g. mast cells among others, which release pro-inflammatory mediators. The mediators activate the peripheral nociceptive terminal by depolarizing it through its receptors, generating an orthodromical action potential as well as the release of the pro-inflammatory substances substance P (SP) and calcitonin gene-related peptide (CGRP). Sensitization of the nociceptors also occurs as a result. IL-1β= interleukin 1β, 5-HT= serotonin, PGs= prostaglandins, NGF= nerve growth factor, TNF-α= tumor necrosis factor α, TRP= transient receptor potential channel, GPCRs= G protein-coupled receptors, NaV= voltage-gated ion (Na) channel, P2X= purinergic receptor, RTK= receptor tyrosine kinase.

When a stimulus is sufficiently noxious and long enough to produce an action potential in a nociceptor, it starts a complex array of reactions first at the peripheral terminal and, depending on if the stimulus is sufficient enough, all the way through the pain pathway (see Figure 1). Nociceptor endings lie adjacent to other cells, like for example keratinocytes, Langerhans cells and mast cells in the skin (Lumpkin and Caterina 2007), with whom they can, and do, interact. As mentioned, nociceptors express different kinds of receptors. The special group of receptors that convert the energy from the noxious stimulus into an action potential, and consequently pain, are called transducers (McMahon et al. 2013). The transducers are activated by different modalities and intensities of stimulus, and many to more than one form of stimulus, e.g. transient receptor potential vanilloid 1 (TRPV1), a rather common receptor, is activated by noxious heat and chemical stimuli (Caterina and Schumacher 1997, Caterina et al. 2000). After the membrane potential has risen above the action potential threshold with the help of transducers alongside voltage-gated ion channels (Basbaum et al. 2009), the action potential is conducted towards the cell soma. At the same time, the action potential might also move anti-dromically (Ferrell and

Russell 1986), i.e. away from the soma, into other peripheral branches of the axon, getting them to release different peptides, like e.g. SP, CGRP, somatostatin (SST) and neurokinins A and K (NKA and NKK) causing a neurogenic inflammation peripherally (McMahon et al. 2013). Anti-dromic activity might also arise from the spinal cord (Sluka et al. 1993).

In the resulting neurogenic inflammation, CGRP is the prime mover for vasodilatation (i.e. hyperaemia), whereas SP and NKA are the main mediators in the (first phase) plasma extravasation (i.e. oedema; in a later stage inflammatory mediators like bradykinin, serotonin (also referred to as 5-HT) and histamine uphold extravasation non-neurogenically (Lischetzki et al. 2001), even though all of these peptides seem to have some role in both neurogenic vasodilatation and extravasation (Holzer 1992). Substance P also induces the accumulation of leukocytes to the inflamed tissue (Walsh et al. 1995). Substance P and CGRP then trigger the release of various inflammatory mediator substances from leukocytes (Holzer 1992) among other cells. Substance P additionally is able to degranulate mast cells, also releasing inflammatory mediators (Hagermark et al. 1978). This inflammatory soup contains mediators like bradykinin, prostaglandins, thromboxanes, cytokines and interleukins, serotonin and histamine from e.g. mast cells, leukocytes, fibroblasts, keratinocytes and platelets (McMahon et al. 2013). What most of the constituents in the inflammatory soup have in common, is that they sensitize the nociceptors (see Hyperalgesia & sensitization) via different manners and receptors, either directly or indirectly. This can happen by for example lowering the nociceptor's threshold for stimuli, like e.g. prostaglandins (England et al. 1996). The mediators mostly act synergistically (histamine potentiates nociceptor response to bradykinin (Mizumura et al. 1995), but may also antagonize one another (activation of histamine H_3 receptors attenuates the release of inflammatory peptides and consequently reduces pain and inflammation (Cannon et al. 2007).

In contrast to the receptors on the peripheral terminal of the nociceptive afferent that transduce and conduct the pain signal forward, there are also those who modulate the signal and work in an anti-nociceptive manner. They belong to the group G-protein-coupled receptors or GPCRs and involve opioid, cannabinoid, SST, α2-adrenergic, muscarinic acetylcholine, γ-aminobutyric acid ($GABA_B$) and metabotropic glutamate receptors (mGluRs) (McMahon et al. 2013). The GPCRs bind to and alter the function of

ion channels (Mark and Herlitze 2000, Pan et al. 2008), whose function is absolutely necessary for neurotransmitter release and signal conduction. The ligands binding to the GPCRs are released from the same cells as the pro-inflammatory mediators mentioned above, e.g. opioid peptides are released from leukocytes (Schafer et al. 1994).

Nociception is the first stop when moving towards a painful experience. As we have seen, it is a rather complex event, with receptors and mediators working to forward the signal to the central nervous system (CNS) at the same time as others try to attenuate the signal and hinder it from continuing.

1.3.2 Dorsal root ganglion & spinal cord

The neuronal cell population in the dorsal horn consists of four different types of neurons; 1) the central terminals of the primary afferent nociceptors, which arborize and terminate in different laminae; 2) interneurons, which send signals inside the spinal cord; 3) projection neurons, which have axons going rostrally through the spinal cord and into the brain; and 4) descending neurons projecting from various areas of the brain, very important in descending pain modulation (McMahon et al. 2013). The different kinds of cells interconnect, forming a very complex neuronal circuitry, e.g. most dorsal horn neurons probably synapse with primary afferents as well as excitatory and inhibitory interneurons (see below) (Todd 2010). The dorsal horn thus works as a two-way street in the pain pathway, relaying pain signals from the periphery to the brain, while modulating the descending pain response. Making it even more complex, non-neuronal cells, i.e. glial cells among others, aid in pain processing and modulation (McMahon et al. 2013). This intricately woven neuronal circuitry in the spinal/trigeminal dorsal horn is yet to be understood completely.

The cell bodies of the nociceptive afferents are located in the dorsal root ganglion (DRG) for afferents innervating the body, and in the trigeminal ganglion for nociceptors innervating the face. Two main axon branches come out of the cell bodies, one projecting peripherally to innervate the target organ, and one central, which projects into the spinal cord or the trigeminal subnucleus caudalis to relay nociceptive signals further up the pain pathway (Basbaum et al. 2009). The afferents enter the spinal dorsal horn and synapse on

second-order neurons in one of 10 distinct laminae, i.e. areas, into which the dorsal horn is divided (Rexed 1952, Molander et al. 1984). The place of the central terminal of the primary afferent in the dorsal horn depends on the type of nociceptor in question (see Figure 2); myelinated A-fibre nociceptors seem to mainly terminate in laminae I, II and V (Light and Perl 1979, Woodbury and Koerber 2003), while C-fibre afferents mainly terminate in laminae I and II, with some terminals dispersed in deeper laminae (III-V) (Silverman and Kruger 1988b, Plenderleith et al. 1990, Averill et al. 1995, Woodbury et al. 2000). Some neurons encode stimuli in the noxious as well as innocuous range and are consequently called wide dynamic range neurons (WDRs). These neurons terminate mainly in deeper laminae (Mendell 1966). The molecules released from neuronal and non-neuronal cells into the spinal cord form a vast and still growing list- with many of the same mediators mentioned at the peripheral nociceptive terminal upon activation- and science has still to shine a light on the exact roles and interactions of all the mediators and transmitters involved in spinal nociceptive modulation. The effect of the released substances can be either anti-nociceptive or pro-nociceptive, or both (McMahon et al. 2013).

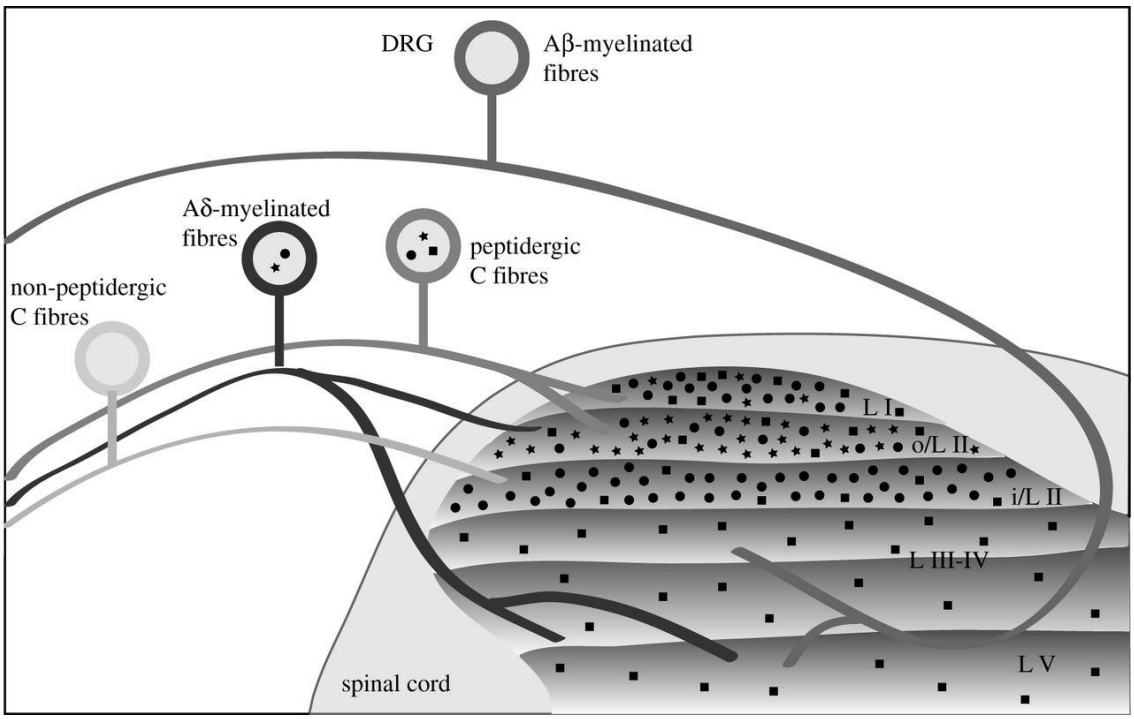

Figure 2. Afferent terminals in spinal cord and principal termination sites. Cell somas are located in the dorsal root ganglion (DRG) and the different neuron types terminate in different laminae (L I-V) of the spinal dorsal horn; peptidergic C-fibres terminate in lamina I and the outer part of lamina II (o/L II), while non-peptidergic C-fibres terminate mainly in the inner lamina II (i/L II). Thin Aδ-fibres mediating pain terminate in laminae o/L II and IV-V and thicker Aβ-fibres terminate mainly in deeper laminae (III-V). Stars represent cannabinoid receptors, circles represent transient receptor potential vanilloid 1 (TRPV1) and squares represent fatty acid amid hydrolase (FAAH), a catabolic enzyme for

cannabinoid receptor ligands. From (Starowicz and Przewlocka 2012)**.**

Interneurons are in majority concerning neural cells in the spinal dorsal horn (Koltzenburg 2000, McMahon et al. 2013). There are two kinds of interneurons; excitatory and inhibitory. The inhibitory interneurons use GABA and/or glycine as their neurotransmitter (Todd and Sullivan 1990, Polgar et al. 2003), whereas excitatory interneurons use glutamate (Yasaka et al. 2010). Inhibitory interneurons have five major tasks to perform; 1) to attenuate the responses of nociceptors to noxious stimuli (Zieglgänsberger and Sutor 1983, Saadé et al. 1985), 2) to silence the neurons in the absence of noxious stimuli (many nociceptive dorsal horn neurons are silent in the absence of noxious stimuli, and therefore need perpetual inhibition to keep them from firing spontaneously) (Cervero et al. 1976, Iggo et al. 1988, Ruscheweyh and Sandkuhler 2003, Schoffnegger et al. 2008), 3) inhibitory interneurons separate different sensory modalities by inhibiting excitatory interneurons that link together low-threshold Aβ-afferents and nociceptive-specific neurons. These excitatory interneurons are normally silent (due to inhibition), but attenuated inhibition could thus lead to pain from otherwise innocuous stimuli (McMahon et al. 2013). 4) Inhibitory interneurons hinder the spread of nociceptive input to other sensory modalities or parts of the body. The sensory afferents in the spinal dorsal horn are organized somatotopically and according to sensory modality (Wilson et al. 1986, Takahashi et al. 2007). Blocking of the $GABA_A$ and glycine receptors in the dorsal horn (i.e. blocking of inhibition) leads to a state, where the excitation of the afferent stimulation site can spread practically anywhere in the dorsal horn (Ruscheweyh and Sandkuhler 2005). 5) Lastly, to prevent too high post-synaptic Ca^{2+}-levels (which consequently lead to easier depolarization) in longer-lasting pain states, inhibitory interneurons hinder a post-synaptic Ca^{2+}-influx either by directly altering the activity of the Ca^{2+}-permeable channel (post-synaptic inhibition) or pre-synaptically by reducing the release of neurotransmitters, which trigger the activity of the Ca^{2+}-channel and thus leads to a Ca^{2+}-influx (McMahon et al. 2013).

Neurons in the spinal cord that connect directly to areas in the brain are called projection neurons. These are found primarily in lamina I of the dorsal horn, as well as scattered across the deeper laminae III-VI and the ventral horn (McMahon et al. 2013). The caudal ventrolateral medulla (CVLM), the nucleus of the solitary tract (NTS), the lateral

parabrachial area (LPB), the periaqueductal grey matter (PAG) and certain thalamic nuclei make up the principal target areas of the lamina I projection neurons in the brain (Gauriau and Bernard 2003). A substantial part of lamina I projection neurons project to more than one supraspinal area (e.g. LPB, PAG & thalamus), which could make for interneuronal differences in function, depending on which area(s) the neuron projects to (Al-Khater and Todd 2009). While most neurons project only contralaterally from their dorsal horn origin, some of them have bilateral projections (Spike et al. 2003). The majority of projection neurons in lamina I are activated by noxious stimuli, even though some are activated by innocuous cold (Willis et al. 1974, Han et al. 1998, Bester et al. 2000, Zhang and Giesler 2005, Andrew 2009). In the dorsal horn, only neurons that respond to noxious stimuli express the neurokinin 1 receptor (NK1R), which is the primary target of SP (Salter and Henry 1991). Studies in the rat spinal cord show that about 80% of lamina I projection neurons express NK1R (Todd et al. 2000, Spike et al. 2003, Al-Khater et al. 2008). Excitatory interneurons have also been shown to express NK1R (Littlewood et al. 1995), but to a vastly lesser extent compared to projection neurons (Al Ghamdi et al. 2009). Targeted ablation of NK1R-expressing cells in lamina I inhibits development of hyperalgesia (see Hyperalgesia & sensitization) in neuropathic and inflammatory pain models (Mantyh and Rogers 1997, Nichols and Allen 1999), which makes these cells highly interesting in the process of chronification of pain. An overview of the cellular interaction in the spinal cord can be seen in Figure 3.

Non-neuronal cells involved in pain processing and modulation in the spinal cord include glial (i.e. microglia, astrocytes and oligodendrocytes) and white blood cells (McMahon et al. 2013). Oligodendrocytes myelinate axons of neurons and have no known role in pain processing in current knowledge (Haydon 2001). Astrocytes make up for about 50% of the glial cell population in the CNS, while microglia make up for some 10-20% (Raivich et al. 1999). Microglia, in their resting state, constitute part of the immune surveillance of the CNS with their macrophage-like function (Eglitis and Mezey 1997, Kurz 1998). Microglia express a wide range of receptors, including receptors for several neurotransmitters, e.g. glutamate and GABA, and activation of different kinds or combinations of receptors consequently lead to different biochemical responses (Noda et al. 2000, Hagino et al. 2004, Kuhn et al. 2004).

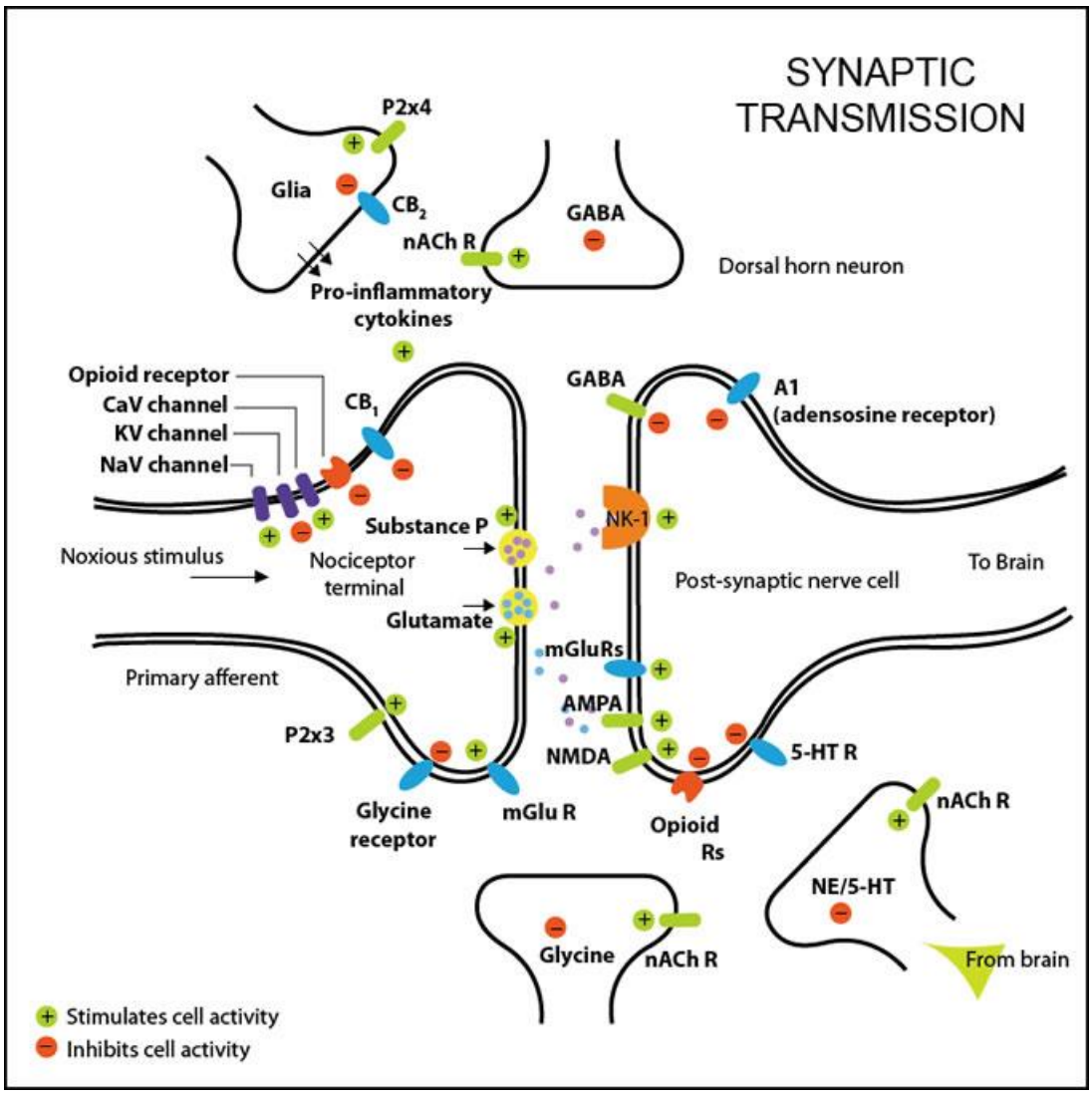

Figure 3. Neuronal and glial interaction in the spinal cord. The net stimulation/inhibition determines if an action potential is generated and sent to supraspinal sites from the post-synaptic nerve cell (projection neuron). P2x3/4 = purinergic receptors, CB = cannabinoid receptors, nACh R = nicotinic acetylcholine receptors, NK-1 = neurokinin 1 receptor, NE = norepinephrine/noradrenaline, mGluRs = metabotropic glutamate receptors, GABA = γ-aminobutyric acid, AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, NMDA = N-methyl-D-aspartate receptor, Ca-/K-/NaV channels = voltage-gated ion channels, 5-HT = serotonin, opioid Rs = opioid receptors. (http://projects.hsl.wisc.edu/GME/PainManagement/session2.2.html, 10.3.2016)

Astrocytes lie tightly adjacent to neurons and microglia, and each astrocyte have contact with thousands of synapses (Bushong et al. 2002). Astrocytes release glutamate into the synapses (Montana et al. 2004, Nadkarni and Jung 2004, Zhang et al. 2004a) and are also primarily in charge of the reuptake of it (Hertz et al. 1978, Minelli et al. 2001), as neuronal glutamate reuptake is deficient. In this manner, astrocytes alter the synaptic activity, and deficits in either release or uptake of glutamate by the astrocytes could thus lead to altered pain states. Since neurons don't possess the enzyme pyruvate carboxylase, which is needed

for the synthesis of glutamate from glucose (Yu et al. 1983, Shank et al. 1985, Kaufman and Driscoll 1992, Gamberino et al. 1997, Waagepetersen et al. 2001), they depend on astrocytes for the production of the neurotransmitter (Halassa et al. 2007). Astrocytes, like microglia, possess a wide array of receptors on their membranes, e.g. GABA (Pastor et al. 1995).

Upon e.g. peripheral nerve injury or inflammation, astrocytes and microglia are activated through neurotransmitters among other mediators. This leads to an increase in cell count for mentioned cells as well as complex intracellular signalling pathways that ultimately lead to synthesis and release of pro-inflammatory mediators like IL-1β, IL-6, TNF-α, prostaglandins and nitric oxide (NO) (Zhuang et al. 2005). The released inflammatory mediators further alter the activity at the synapses that the glial cells connect to, as well as the expression of membrane receptors on glial cells. Glutamate reuptake from the synapses is also decreased as a consequence of activation, which has an excitatory effect on the affected synapses. As astrocytes stay activated even during prolonged states of nociceptive input, it seems probable that they could play a role in generating and maintaining chronic pain (Sung et al. 2003, Tawfik et al. 2006, Ru-Rong and Suter 2007). Microglia also exert anti-inflammatory effects while activated, by clearing dying and damaged cells and cellular debris by phagocytosis (De Simone et al. 2004) and synthesizing and releasing anti-inflammatory mediators (Hacker et al. 2006), like interleukin 10 (IL-10) (Olson and Miller 2004).

White blood cells (WBCs) are normally scarce in the CNS, but following peripheral nerve injury, chemokines released from e.g. neurons or glial cells direct leukocytes to central terminals of the injured nerve (Fabry et al. 1995, Mark and Miller 1999). However, WBCs mainly seem to contribute to the hyperalgesia present in the state of neuropathic pain following nerve injury (Cao and DeLeo 2008, Costigan et al. 2009).

Nociceptive processing and modulation in the spinal cord is also influenced by descending monoaminergic pathways originating in the brain (Reynolds 1969, McMahon et al. 2013). The axons projecting from supraspinal sources may contain and release mainly serotonin, noradrenaline or dopamine (Fuxe 1965, Commissiong et al. 1978, Bowker et al. 1981).

Descending axons can modulate pain transmission by stimulating the terminals of primary afferents, projection neurons, inhibitory or excitatory interneurons or other descending neurons in the spinal cord (Millan 2002). They can also exert their effect on non-neuronal components in the dorsal horn, like e.g. astrocytes (Jalonen et al. 1997) and modulate pain indirectly through them. Descending pain modulation can be inhibitory or excitatory in nature, depending on which receptors and receptor-subtypes the neurotransmitters bind to, since some receptors or their subtypes mediate descending inhibition, while others facilitate nociceptive transmission (Zemlan et al. 1983, Bobker and Williams 1989, Zhuo and Gebhart 1990, Zhuo and Gebhart 1991). The supraspinal sites from where the descending neurons project will be discussed in the next chapter.

1.3.3 The brain and brainstem

Pain-associated neurons projecting from the spinal cord to supraspinal targets are organized in bundles, thus creating different pathways. These include- as far as we know today- mainly the spinothalamic (STT) and the spinobulbar & -medullary pathways (McMahon et al. 2013). Other, less pronounced pathways have also been identified (e.g. spinohypothalamic and spinocervicothalamic pathways and the post-synaptic dorsal column system), but the specifics of these are yet to be defined. The neurons in some of the bundles are- and continue to be during ascension to and termination in supraspinal targets- topographically organized, while some are more disorganized in this manner (McMahon et al. 2013). While many cells projecting through these pathways originate in the superficial or deep dorsal horn, neurons from the ventral horn of the spinal cord also join in. The different pathways, consisting of neurons encoding noxious as well as innocuous stimuli and terminating either directly or indirectly in various parts of the brain or brainstem, thus are thought to be responsible for the multiple aspects of pain (e.g. sensory, emotional) (McMahon et al. 2013). There is also evidence of cross-activation between separate pathways (Djouhri et al. 1997). Species differences in the organization of the pathways and the termination of the neuronal cells in the brain have been shown to exist and even be quite extensive between some species (McMahon et al. 2013).

The spinothalamic pathway, which- as the name suggests- ascends from the spinal cord to the thalamus (Th), is the one most extensively studied and most important spinal-

supraspinal pathway considering pain and temperature sensation (Trevino et al. 1973, McMahon et al. 2013). The pathway originates in three regions within the spinal cord; the superficial dorsal horn lamina I, the deep dorsal horn laminae IV-V and the medial ventral horn laminae VII-VIII (Trevino et al. 1973). The different groups consist of cells with differing afferent input and consequently functional activity (Christensen and Perl 1970). The lamina I neurons constitute nearly 50% of the cell population of the STT, while the other groups make up about 25% each (McMahon et al. 2013).

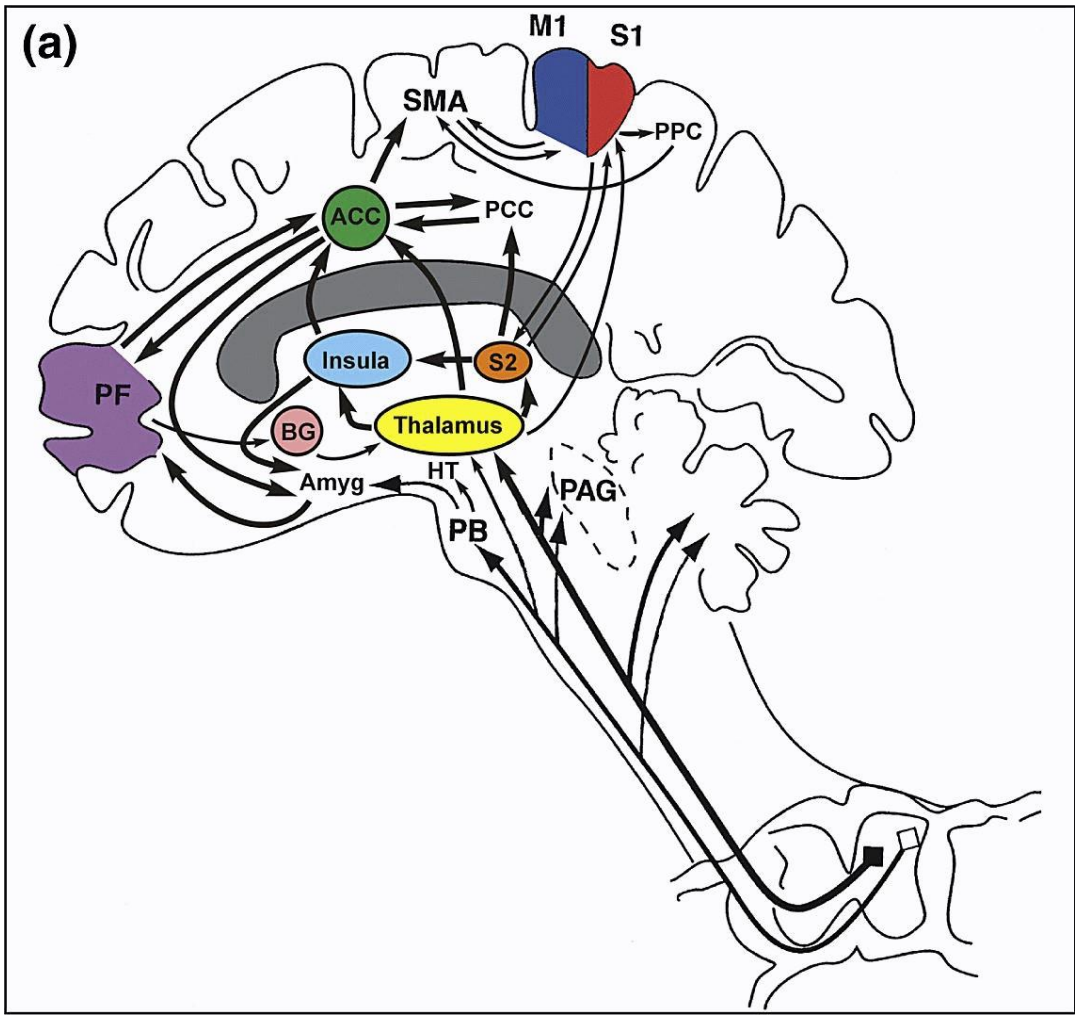

Figure 4. Ascending projections to brain areas indicated to be involved in nociception. Notice the contralateral ascent of projection neurons. PB= parabrachial nucleus, PAG= periaqueductal gray, HT= hypothalamus, Amyg= amygdala, BG= basal ganglia, ACC= anterior cingulate cortex, PCC= posterior cingulate cortex, PPC= posterior parietal cortex, M1 and SMA= primary and supplementary motor cortices, S1 and S2= primary and secondary somatosensory cortices, PFC= prefrontal cortex. From (Apkarian et al. 2005)**.**

The lamina I STT-neurons mainly include three different types of cells; 1) nociceptive-specific neurons with input mainly from Aδ-fibers, 2) polymodal nociceptive neurons with input mainly from C-fibre afferents and 3) neurons activated by innocuous thermal stimuli

(Craig 2003). The vast majority of lamina I STT-neurons project to the contralateral thalamus, with only a fraction projecting ipsilaterally (Carstens and Trevino 1978, Willis et al. 1979). It's been concluded that the lamina I nociceptive-specific and polymodal nociceptive neurons are associated with first, fast-onset sharp pain and second, slower-onset, burning pain, respectively (Andrew and Craig 2002, Craig and Andrew 2002). The STT group of laminae IV-V neurons receive their input mainly from Aβ-fibers from the skin, although many also have monosynaptic input from nociceptive Aδ-fibres as well as polysynaptic input from C-fibres, the latter ones originating in the skin as well as deeper tissues. While some neurons of the group are activated by low-threshold (innocuous) mechanical stimuli or high-threshold (noxious) mechanical or heat stimuli, most neurons respond to both, i.e. they are WDRs (McMahon et al. 2013). Lamina V neurons have been proven to be involved in motor reflex activity, like withdrawal reflexes in response to painful stimuli (Schouenborg et al. 1995). The neurons projecting from laminae VII-VIII are large cells which transmit noxious and innocuous stimuli from skin as well as deeper tissues (Meyers and Snow 1982). They possess large somatic receptive fields (Meyers and Snow 1982) and may be excited or inhibited by various somatic input (e.g. stimuli regarding proprioception or the viscera) (Giesler et al. 1981). The different cell groups terminate in different nuclei of the thalamus (discussed later) (Craig 2003).

Spinobulbar projections ascend to the brain stem to regions regulating homeostasis and behavioural state and some also continue to higher brain centers (Craig 2003). Cells in the spinobulbar tract are distributed in the spinal cord in a fashion similar to that of STT-cells, i.e. they arise mainly from laminae I, V and VII (Wiberg et al. 1987). The response characteristics of the spinobulbar cells also are quite alike those of spinothalamic cells (Yezierski and Schwartz 1986, Ammons 1987, Wilson et al. 2002). The spinobulbar neurons have their termination sites predominantly in four major areas of the brain stem; the catecholamine cell group region, the parabrachial nucleus (PB), the periaqueductal gray (PAG) and the brain stem reticular formation (Wiberg et al. 1987, Craig 2003). Lamina I neurons ascend to the catecholamine cell groups, the PB and the PAG, but not to the reticular formation (Craig 2003), whereas laminae V and VII neurons primarily project to the reticular formation as well as the lateral reticular nucleus and the tectum with sparse projections to the PB, the PAG and the catecholamine cells (Yezierski 1988, Andrew et al. 2003). Figure 4 shows some projections to and activation of brain areas known to be

activated by nociceptive stimuli.

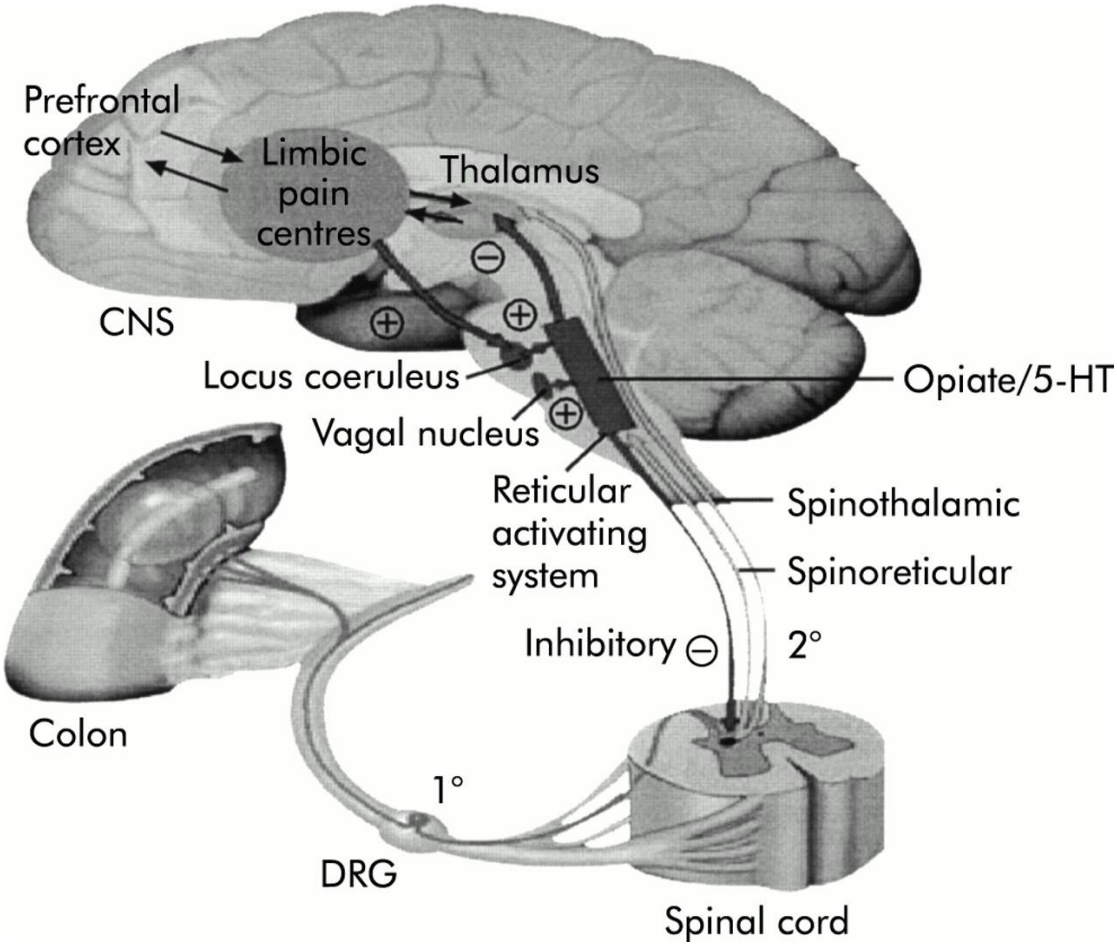

Figure 5. Overview of the nociceptive network including the inhibitory descending pathway. + stands for stimulation and – for inhibition. 1˚= first order neuron, 2˚= second order neuron, CNS = central nervous system, DRG = dorsal root ganglion, 5-HT = serotonin. (http://neuroanatomyblog.tumblr.com/image/27908577874, 10.3.2016)

The catecholamine groups, which include the locus coeruleus, the ventrolateral medulla and the nucleus of the solitary tract among other nuclei, are an integral component of homeostatic and cardiorespiratory function (Sato and Schmidt 1973, Craig 2003). Activation of these groups by means of stressful situations, like e.g. pain, may result in activation of the hypothalamus (Craig 2003) and/or somato-autonomic spino-bulbo-spinal reflex arcs modulating homeostasis (Sato and Schmidt 1973) and descending modulation of nociception (inhibition or excitation) (Millan 2002). The PB cells serve as an integral component for nociceptive and general visceral afferent activity. They also conduct information indirectly to forebrain autonomic, neuroendocrine and emotional control areas (McMahon et al. 2013). The PB cells interconnect with reticular formation and catecholamine group cells, supposedly as part of maintenance of homeostasis (Chamberlin

and Saper 1992), and they project to several regions in the brain including the hypothalamus, amygdala and the thalamus, which relays the insular cortex (McMahon et al. 2013). The PAG is an essential mesencephalic part in controlling homeostasis and limbic motor output and it has both ascending and descending projections (Bandler et al. 2000). Stimulation of the PAG may result in aversive behaviour, cardiovascular changes and opioid or non-opioid-mediated analgesia (Bandler et al. 2000). PAG plays a major role in descending analgesia by means of its projections to the nucleus raphe magnus (NRM) in the rostral ventromedial medulla (RVM), pons and medulla (Basbaum and Fields 1978, Millan 2002). Especially the descending connection from the PAG to RVM is essential, since major output from the PAG to the spinal cord goes via the RVM and lesions in or inactivation of the RVM results in attenuated analgesia after PAG stimulation (Fields et al. 1991, McMahon et al. 2013). The RVM plays a major role in descending modulation (inhibition) of pain, not only because of the input from the PAG, but because of the cell populations that inhabit it (Fields and Heinricher 1985, Millan 2002). Three distinct groups of neurons can be characterized based on their reaction to noxious heat prior to the withdrawal reflex; ON-cells discharge just before the reflex; OFF-cells stop their discharge prior to the reflex; NEUTRAL-cells show no consistent change in firing at the withdrawal reflex (Fields and Heinricher 1985). Modulation of pain depends on the net firing; more ON-cells firing leads to facilitated nociception while OFF-cells firing in majority leads to attenuated nociception (McMahon et al. 2013). The parts of the PAG receiving spinal input have been shown to ascend further to the hypothalamus and thalamus (Mantyh 1983). The cells in the reticular formation play a role in the motivational-affective as well as autonomic responses to painful stimuli (Almeida et al. 2004).

The thalamus is the main relay station for nociceptive stimuli reaching for cortical sites, and it is involved in reception, integration as well as transfer of the stimuli, and it is in this part of the pain pathway that the affective-motivational and sensory-discriminative components of the pain experience are integrated in the painful stimulus. The thalamus receives projections to its several nuclei from many sources (e.g. STT, PAG), and in turn have a vast network of projections to cortical (e.g. somatosensory cortices) as well as subcortical (e.g. HT, PAG, amygdala) regions of the brain. The wide array of interconnections of the thalamus puts it at the centre of the intricate pain processing system that is the brain (Almeida et al. 2004, Yen and Lu 2013).

Cortical structures most consistently activated in imaging studies of pain include the prefrontal cortex (PFC), the anterior cingular cortex (ACC), the insular cortex (IC) and the primary and secondary somatosensory cortices (S1 & S2). Encoding of the nociceptive stimulus in these areas leads to the complex pain experience. The input reaching these somatosensory (S1, S2 and IC), limbic (IC, ACC) and associative (PFC) regions of the brain stems from several nociceptive pathways as described before (Apkarian et al. 2005). Figure 5 shows an overview of the whole pain system from nociception to perception and modulation.

1.4 Plasticity of the pain pathway

The complex pain encoding network is a highly plastic one, constantly encoding noxious stimuli and reacting to it based on e.g. the length or the intensity of the stimuli. The body responds to noxious stimuli, e.g. wound injury, by modulating the incoming stimuli both locally and centrally. The modulation might be either pro- or anti-nociceptive, but more often is pro-nociceptive.

1.4.1 Hyperalgesia, allodynia & central sensitization

Following an injury/nociceptive response, the injured area and its surroundings become hyperalgesic (Lewis 1935). Hyperalgesia, as the name suggests, is defined by the IASP as ”increased pain from a stimulus that normally provokes pain” (International Association for the Study of Pain 2012) (i.e. suprathreshold stimuli to high-threshold nociceptors). Hyperalgesia at the site of the injury is called primary hyperalgesia, as opposed to secondary hyperalgesia, which is hyperalgesia of the uninjured but injury-adjacent tissue (Lewis 1935). Primary hyperalgesia usually develops for heat and mechanical stimuli (Raja et al. 1984), but may vary depending on the specific tissue in question (Campbell and Meyer 1983). Primary hyperalgesia is, at least partly, driven by changes in peripheral nociceptors that have become sensitized (Meyer and Campbell 1981), leading to e.g. lowered thresholds, augmented responses to suprathreshold stimuli and expanded receptive fields (Thalhammer and LaMotte 1982, Raja et al. 1984, Reeh et al. 1987, Cooper et al. 1993).

Secondary hyperalgesia, which develops in the area surrounding injury, is a phenomenon arising from the CNS (Treede et al. 1992). The area of secondary hyperalgesia becomes sensitized to mechanical stimuli, but not to heat stimuli. In fact, stimulus-responses to heat stimuli in the area might be attenuated, making it hypoalgesic to heat-stimuli (Raja et al. 1984, Ali et al. 1996). Enhanced responsiveness, i.e. sensitization of nociceptors in the case of secondary hyperalgesia thus is due to sensitization of CNS-neurons relaying noxious stimuli, not peripheral nociceptors (Simone et al. 1991). Secondary hyperalgesia, or primary mechanical hyperalgesia for that matter, can further be divided into punctate and stroking hyperalgesia, which arise through different neural mechanisms, where punctate hyperalgesia is the result of sensitization of nociceptors in the CNS (LaMotte et al. 1991).

Stroking hyperalgesia, also termed allodynia, is ”pain due to a stimulus that does not normally provoke pain” (International Association for the Study of Pain 2012) and an altogether different form of pain generation. Whereas punctate and heat hyperalgesia stem from the sensitization of nociceptors, allodynia originates in low-threshold mechanoreceptors that normally responds to innocuous touch-stimuli. These low-threshold Aβ-fibres are integrated into the pain network because of central sensitization, thus enabling a normal touch sensation to become painful (Koltzenburg et al. 1992, Torebjork et al. 1992, Seal et al. 2009).

Central sensitization is a complex and important phenomenon especially in chronic pain disorders, e.g. in neuropathic pain states (Woolf 2011). Central sensitization is defined as ”increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input” (International Association for the Study of Pain 2012). In order for central sensitization to arise in the CNS, sensory input to peripheral terminals, i.e. activation of the pain pathway, is required (LaMotte et al. 1991, Torebjork et al. 1992). Input to the pain network, e.g. injury to the skin, strengthens the synaptic activity in the spinal cord nociceptive neurons and this lasts for at least several minutes after the end of the noxious stimulus (Woolf 1983, Woolf 1991, Treede et al. 1992). The augmented synaptic transmission occurs in the very neurons that are activated in the dorsal horn (homosynaptic potentiation or wind-up) (Mendell 1966, Woolf and Swett 1984, Dickenson and Sullivan 1987) as well as in non-activated nociceptive and non-nociceptive neurons (heterosynaptic potentiation) in both the ventral and the dorsal horn of the spinal cord (Thompson et al. 1993). The increase in synaptic activity may be due to higher membrane excitability or an increased release of neurotransmitter pre-synaptically and/or an increased

response to the neurotransmitter post-synaptically (Woolf and King 1990, Woolf and Thompson 1991, Thompson et al. 1993, Wang et al. 2005, Li and Baccei 2009, Tao 2010) as well as a reduced level of inhibition in the spinal cord (Sivilotti and Woolf 1994, Moore et al. 2002, Baba et al. 2003, Miraucourt et al. 2009). A major component for the induction and persistence of central sensitization is the activation of NMDA-receptors (Woolf and Thompson 1991). Antagonism of the NMDA-receptors in turn diminishes the centrally sensitized state (Woolf and Thompson 1991).

As most synaptic input normally is subthreshold (Woolf and King 1987, Woolf and King 1989) and thus doesn't evoke an action potential, with the changes described above the input now might elicit a response in form of an action potential and subsequent activation of nociceptive pathways that otherwise wouldn't be activated from that particular stimulus, leading to changes in both the pain network and the sensation of pain (Woolf et al. 1994). As we can see, central sensitization is not merely a threshold-lowering process, but a modality-changing (touch to pain) entity which alters the basic function of pain, which also can be seen as changes in activity in the cortical areas involved in the brain (Maihofner et al. 2006). The phenomenon of central sensitization is normally transient in nature, i.e. subsequent activation of spinal cord nociceptors is required for it to persist, or the responsiveness of the nociceptors normalizes (Cook et al. 1987). However, in some pathological pain states, e.g. dysfunctional pain in fibromyalgia, the state of central sensitization is persistent even without sensory input to the pain pathway, making the individual chronically painful (Wolfe et al. 1990, Gibson et al. 1994, Lorenz et al. 1996).

2 ACUPUNCTURE

Acupuncture is a series of techniques used to treat illnesses and usually involves the use of needles (Ulett et al. 1998). Acupuncture is best known as part of traditional Chinese medicine (TCM) practices, even though there is early evidence of people using acupuncture-related techniques to treat disease also outside Asia, e.g. Brazil, Africa, the Eskimos (Gori and Firenzuoli 2007). Acupuncture is thought to have been used and developed in China for some 3000 years (Schoen 2001). The first depiction of acupuncture in Western medicinal literature stems from circa 1680 by the Dutch physician Ten Rhijne

(Baldry and Thompson 2005). The interest for Eastern medicine and acupuncture grew quite rapidly among European and American physicians during the first half of the 19th century, only to be left dormant for about a century. The latter half of the 20th century witnessed the ”comeback” of TCM and especially acupuncture in Western medicine. Since then, particularly during the last couple of decades, extensive, evidence-based research into the neurophysiology and use of acupuncture has been carried out by means of Western research standards and the popularity of acupuncture on the Western hemisphere keeps on growing (Schoen 2001, White and Ernst 2004). The World Health Organization (WHO) has accepted acupuncture as an effective treatment method for some pain conditions (e.g. low back pain) based on clinical trials (World Health Organization WHO 2002). In the next chapter I will present some basic principles of acupuncture including some comparisons between TCM and Western medicine (WM) and more importantly, the neurophysiologic mechanisms behind the efficacy. According to TCM nearly any disease can be treated with acupuncture, but as most of the research to date is focused on the analgesic effect of acupuncture, I too will concentrate on the process resulting in attenuated pain sensation.

2.1 Basic principles of acupuncture

Acupuncture treatment is based on the stimulation of acupuncture points or acupoints. According to TCM, most acupuncture points reside along 14 main meridians. 12 of these meridians are thought to regulate, communicate with and reflect the status of visceral organs. The meridians are organ-specific, e.g. Kidney, Spleen and Lung and these are bilateral. The remaining two major meridians are located along the dorsal and ventral midline respectively (Schoen 2001). Though some organs and their meridians share the same name, e.g. liver, one cannot equalize the liver in WM to that of TCM. Whereas an organ in WM is based on its anatomy, structure and function, organs in TCM are defined only by their function with only some, if any, relations to anatomy. This makes the TCM organ systems difficult to extrapolate to WM and therefore also TCM-treatments hard to understand in a WM perspective (Kaptchuk 2000).

In TCM philosophy, there are two opposing and complementary forces, Yin and Yang, coexisting in nature. These forces act together to regulate the flow of the ”vital force”, also known as Qi. When an individual is healthy, Yin and Yang are in balance compared to each other, and the flow of Qi is smooth and regular (Kaptchuk 2000). On the other hand,

imbalance of Yin and Yang lead to disturbances or obstruction in the Qi-flow and consequently illness or disease. Qi is thought to flow through the meridians from the internal organs to the skin. Stimulation of acupoints (see below) along the meridians with faulty Qi-flow is supposed to restore balance between Yin and Yang and normalize Qi-flow thus returning the body to good health (Kaptchuk 2000, Wang et al. 2008).

2.2 Acupoints

According to TCM-teachings, specific points residing along the meridians reflect the condition of the visceral organs. These points are generally called acupuncture points or acupoints (Kaptchuk 2000). Some research has been done into the specificity of acupoints in regards to function, structure and characteristics, but the findings as of yet have been inconclusive. No evidence has been found that all acupoints would show any (uniform) specific features that differ from other tissues, although one should keep in mind that the research done on this subject still is quite limited and the existence of specific acupoints, according to WM, still a matter of controversy (Ramey 2001, Ernst 2006, Zhao et al. 2012, Li et al. 2015).

The anatomical studies on acupuncture points have gathered some evidence that acupoints would contain higher densities of nerve endings and neural and vascular structures (Hwang 1992, Li et al. 2004, Zhu et al. 2004, Wick et al. 2007, Zhang et al. 2011a). Mast cells have also been proposed to occur at higher concentrations at acupoints when comparing to other tissue/non-acupoints and it seems acupoint stimulation instigates the degranulation of these mast cells, leading to subsequent activation of other cells (Hwang 1992, Zhang et al. 2008). Connective tissue has been proposed and discussed as a structural and functional component in acupoints, and indeed, one study showed an 80% correlation between the location of intermuscular or intramuscular connective tissue and the sites of acupoints (Langevin and Yandow 2002). Some evidence of correlation between myofascial trigger points (MTrPs) and acupoints has also been found; between 71% and 99.5% of acupoints corresponded to MTrPs through clinical indication of pain (Melzack et al. 1977, Dorsher 2008) and MTrPs also have been proposed as a mechanism for musculoskeletal pain (Melzack et al. 1977, Ge et al. 2008).

The electrical characteristics of acupoints have been a subject of interest during recent years. While some studies have found significantly low impedance in the skin at acupoints

compared to the skin at non-acupoints in healthy test subjects (Zhang et al. 2004b, Silberstein 2009), others found no correlation between acupoints and skin resistance (Pearson et al. 2007, Wei et al. 2012). One study found that acupoints have an either lower or higher impedance than do non-acupoints (Kramer et al. 2009), which would concur with the notion of Qi deficient or Qi excessive acupoints. Another concluded that the impedance in the skin at acupoints along the Lung-meridian in asthmatics was significantly higher than that of healthy controls (Ngai et al. 2011). A review on the topic found that in 5 out of 9 studies, a significant correlation between low skin impedance and acupoints was reported, while the remaining 4 studies could not find a definitive correlation (Ahn et al. 2008). However, the review pointed out that the research-quality of the studies carried out on the matter was quite low, even for the studies included in the review. Therefore, a conclusive correlation between skin electrical characteristics and acupoints remains to be found, even though research points towards a correlation. While a definitive conclusion on the matter awaits, measuring skin impedance is used as a way to locate acupoints and even diagnose disorders (Falk et al. 2000, Ngai et al. 2011, Turner et al. 2013).

Another intriguing acupuncture-related phenomenon is acupoint sensitization as a reflection of visceral disorders (Li et al. 2013). Studies have found either elevated temperatures or pain-sensitization at acupuncture points following visceral disease (Kwon et al. 2007, Li et al. 2013). This phenomenon might be explained by ways of WM in referred pain. Referred pain from visceral organs often lead to hyperalgesia in skin and muscle as well as segmental muscle contracture (Giamberardino and Vecchiet 1995, Morrison et al. 1995, Verne et al. 2003). The theory is, that continuous stimulation of visceral nociceptive afferents in states of disease lead to a sensitization of neurons in the dorsal spinal horn and even supraspinal nuclei, creating hypersensitized sites. Some peripheral (skin/muscle) and visceral afferents converge in the dorsal horn and thus end in the same segment and area of the dorsal horn. These convergent peripheral afferents have been shown to become sensitized following sensitization of visceral afferents. This in turn causes e.g. dermal hyperalgesia and could thusly be an explanation of acupoint sensitization (Garrison et al. 1992, Giamberardino et al. 1996, Roza et al. 1998, Li et al. 2013). Even though it has been shown that analgesia generated by acupuncture is most efficient when stimulating nerves ending in the same spinal segment as the nerves generating pain, many acupoints distant to the site of pain are effective in alleviating it (Wu et al. 1974, Bing et al. 1990, Zhu et al. 2004).

Whereas no compelling evidence for a specific anatomic or biochemical structure for acupoints has been found, it may be that the acupoints differ from other tissues simply by means of functionality; the response intensity of acupoints is differerent from that of other tissues, ergo the distinction between acupoints and other points could be in the degree of response (Cheng 2009).

2.3 The acupuncture pathway

2.3.1 Peripheral tissues

What actually happens following the needle insertion through the skin and into an acupoint? Early research showed an increase in the pain threshold following acupuncture (Chiang et al. 1973). This effect, however, was not seen after application of a local anaesthetic to the deeper muscular layer of the acupoint, whereas blockade of the superficial cutaneous nerves did not block the effect (Chiang et al. 1973). These early results concluded that an intact neural pathway must be present for acupuncture to be able to exert its analgesic effects (Chiang et al. 1973). Subsequent research has affirmed this and specified that intact nociceptive pathways are the essential part for acupuncture to induce analgesia (Pan et al. 1997).

Following insertion and manipulation (twisting and twirling up and down) of the needle into an acupoint a feeling of soreness, numbness, heaviness or distension might occur (Zhao 2008). This feeling, called De-Qi according to TCM, is suggested to be essential for the efficacy of acupuncture analgesia (Wang et al. 1985, Haker and Lundeberg 1990, Hui et al. 2005). The origin of the sensation has been proposed to be impulses from muscles following acupuncture stimulation, especially since a study found the sensation to be abolished after a local anaesthetic was injected into the deeper tissues of the acupoint (Shen et al. 1973). Other deeper tissues have not been ruled out, but the activity of polymodal-type receptors in deep tissues have in fact been proposed to play a key role in the sensation (Kawakita et al. 2002). More recently, connective tissue has been suggested as playing a role in the De-Qi-feeling by signalling to the CNS (Langevin et al. 2001, Langevin and Yandow 2002), as have mast cells, seeing as the densities of mast cells are clearly larger at acupoints comparing to non-acupoints and the analgesic effect is markedly attenuated by the inhibition of mast cell degranulation prior to needle insertion (Zhang et

al. 2008).

The needle penetrating the skin and deeper tissues at the acupoint asserts mild mechanical stimulation activating A-type fibres (Aβ- and Aδ-fibres), with local injuries in deeper tissues leading to the release of different inflammatory mediators such as histamine, adenosine triphosphate (ATP), 5-HT and bradykinin, activating nearby nociceptors either directly or indirectly (Zhao 2008, McMahon et al. 2013). Activation/degranulation of mast cells by mechanical stimulation releases adenosine among other compounds (Yao et al. 2014). Although adenosine has been known for a long time, it is only quite recently that its role as a signalling molecule was elucidated and accepted (Bodin and Burnstock 2001). Adenosine directly activates sensory nerves through purinergic receptors (Yao et al. 2014). The peripheral opioid system acts to attenuate inflammatory pain (Stein 1991, Stein et al. 2003) and studies show that peripheral release of opioids are involved in the generation of EA analgesia (Sekido et al. 2003, Zhang et al. 2005).

It seems that C-fibre activation is involved in and even essential for analgesia by traditional manual acupuncture (MA), while analgesia induced by electroacupuncture (EA), i.e. stimulating currents lead through needles in acupoints, seems to be based upon the activation of Aβ- and Aδ-fibres mainly (Zhao 2008). Concurrent use of both MA and EA provides more potent analgesia than single use of one or the other (Kim et al. 2000).

2.3.2 Spinal cord

The impulses generated by the acupuncture needle (or EA) move towards the spinal cord, where nerves from the same level of the body end in the same spinal cord segment (see also 2.2 Acupoints) (Zhao 2008). The impulses into the spinal cord triggers the release of different neurotransmitters much like an nociceptive impulse would, leading to activation of a variety of spinal cord neurons (Wang et al. 2008) and subsequent transmitting of the signal to higher centres in the CNS, mainly through the ventrolateral funiculus (VLF) in the spinal cord. The VLF also happens to be the spinal pathway for noxious and temperature sensation, again proving the convergent acupuncture and pain signalling pathways (Chiang et al. 1975, Zhao 2008). While descending inhibition acting in the spinal cord is a major part of acupuncture analgesia, this chapter will focus on the ascending acupuncture signals and descending inhibition will be discussed in the next chapter.

Opioid receptors (μ-, δ- and κ-receptors) are widely distributed at peripheral afferent terminals and in pain-related areas of the CNS and are closely involved in anti-nociception (McMahon et al. 2013). An early study in 1973 investigated the analgesic effect of acupuncture by treating rabbits with acupuncture, and then infusing cerebrospinal fluid (CSF) from them into the lateral ventricle of rabbits that had not received acupuncture treatment. The pain thresholds of the recipient rabbits were increased whereas no increase in thresholds were seen in the controls who had received either saline or CSF from non-acupuncture rabbits (Research Group of Acupuncture Analgesia 1974). Following studies found that acupuncture analgesia could be abolished by the opioid-antagonist naloxone and soon researchers also recognized an increase in endogenous opioid-levels in the CSF following acupuncture treatment (Pomeranz and Chiu 1976, Mayer et al. 1977, Sjölund et al. 1977). We now know that the endogenous opioid release constitutes of enkephalins, dynorphin, endomorphin and β-endorphin, and the stimulation frequency in electroacupuncture (EA) affects the proportion in which the opioids are released, e.g. low-frequency stimulation (2 Hz) leads to higher proportions of enkephalin, endomorphin and β-endorphin, whereas high stimulation frequency (100 Hz) results in high levels of dynorphin (Fei et al. 1987, He and Han 1990, Han et al. 1999). Endogenous opioid release is perhaps the most widely known and accepted mechanism of acupuncture analgesia (Peets and Pomeranz 1978, Clement-Jones et al. 1980, Lee and Beitz 1993, Han 2003, Fry et al. 2014). Repeated treatment has been shown to cause tolerance to EA analgesia, and is thought to be mediated by down-regulation of opioid receptors as well as anti-opioid substances (Han et al. 1979b, Han et al. 1981). Thus, opioid-mediated analgesia is an essential part of acupuncture (and EA) analgesia, especially in the CNS (Zhao 2008).

Afferent nociceptive terminals contain large amounts of excitatory amino acids like glutamate and the superficial dorsal horn of the spinal cord is densely populated with their receptors, such as the NMDA-receptor (Liu et al. 1994, Li et al. 1997). As we know, these receptors (especially NMDA) play a major role in both physiological pain processing and transmission as well as in pathological states such as central sensitization in chronic pain (see chapters 1.3.2 and 1.4.1). Studies have shown that the expression of NMDA-receptors in the spinal dorsal horn was attenuated by EA in inflammatory (Choi et al. 2005a, Choi et al. 2005b) and neuropathic pain models (Sun et al. 2004). In another neuropathic pain model, EA attenuated mechanical allodynia when given on its own. However, when EA was given together with a NMDA receptor antagonist, the anti-allodynic effect was clearly

enhanced (Huang et al. 2004). An inflammatory pain model found similar results; while NMDA (or AMPA) receptor antagonists given intrathecally had no effect on thermal hyperalgesia following inflammation when administered alone, they significantly potentiated the effect of EA to the hyperalgesia (Zhang et al. 2002b, Zhang et al. 2003). Interestingly, the same effect has not been found in studies on normal rats, where the administration of NMDA receptor antagonists prior to EA has impaired EA analgesia, suggesting different mechanisms in altering NMDA receptor mediated signalling in the spinal cord in normal and painful animals (Choi et al. 2005b, Kim et al. 2012).

We know that spinal glial cells interact with neurons as a part of spinal pain processing and as it seems, glial cells in the spinal cord (mainly astrocytes and microglia) have a part to play in generating and maintaining a state of chronic pain (Milligan and Watkins 2009). An animal model for inflammatory pain showed that a glial metabolic inhibitor administered intrathecally by itself did not alter the thermal hyperalgesia or mechanical allodynia, whereas electroacupuncture alone reduced the levels of the aforementioned. Concomitant administration of EA and the glial metabolic inhibitor, however, had a significantly elevating effect on EA analgesia (Sun et al. 2006). A similar study done with minocycline, a microglial inhibitor, found that both EA and minocycline alone reduced nociceptive hypersensitivity and microglial activation significantly, and the EA analgesic effects were markedly potentiated when given simultaneously with minocycline (Shan et al. 2007). A recent study in a neuropathic pain model by spinal injury found that acupuncture greatly alleviated pain levels and effectively inhibited microglial activation in the spinal cord (Choi et al. 2012).

2.3.3 Effects in brain and brain stem

Multiple brain areas have been proposed to play a role in acupuncture signalling and analgesia, such as the RVM (mainly NRM), PAG, locus coeruleus (LC), arcuate nucleus (Arc), preoptic area (Po), centromedian nucleus (CM), nucleus submedius (Sm), habenular nucleus (Hab), nucleus accumbens (Ac), caudate nucleus (Cd), amygdala, ACC, and hypothalamic paraventricular nucleus (PVH) (Bing et al. 1991, Takeshige et al. 1991, Yang et al. 1992, Takeshige et al. 1993, Guo et al. 1996, Hui et al. 2005, Yan et al. 2005). The hypothalamic – pituitary – axis (HPA) also is activated following acupuncture, possibly playing a role in acupuncture analgesia (Pan et al. 1994, Pan et al. 1996). While the exact

interactions between the areas in this intricate network are not fully understood yet, studies have made some conclusions regarding the most important activated areas, e.g. hypothalamic activation including the Arc as part of activation of the descending inhibition system (Yu and Han 1989, Wu et al. 1999). Furthermore, it seems that brain activation patterns following acupuncture differ between chronically painful and healthy subjects (Napadow et al. 2007). At the moment it has been proposed that acupuncture activates mainly somatosensory areas in the brain while deactivating the limbic system (Hui et al. 2010). Figure 6 shows a schematic of brain areas commonly activated by acupuncture stimulation.

Many of the brain regions processing acupuncture signals express opioid peptides, like for example the Cd, Ac, Arc, PAG and NRM, where inactivation of opioid receptors lead to decreased analgesic effect following acupuncture (He 1987, Zhao 2008). Early work concluded that the PAG contain opioid receptors and plays a major role in producing analgesia and we now know it is an important part of the descending inhibitory system from the brain to the spinal cord (Tsou and Jang 1964, Reynolds 1969, McMahon et al. 2013). Further studies revealed that EA analgesia was attenuated or potentiated, respectively, when opioid receptor antagonists or compounds preventing the degradation of opioid peptides were injected into the PAG, linking EA analgesia to the PAG in part (Xie et al. 1983, Han et al. 1984, Kishioka et al. 1994). The PAG and the NRM in the RVM are known to be interconnected so that stimulation of the PAG leads to increased firing of the NRM neurons, leading to analgesia (Zhao 2008). A study in rats showed that EA leads to activation of NRM neurons, producing analgesia, but following micro-injection of an opioid receptor antagonist into the PAG, this analgesia was attenuated (Liu 1996). The arcuate nucleus also seems to be involved in this axis, as activation of NRM neurons by EA was further potentiated by Arc stimulation and this could be reversed using naloxone, an opioid antagonist (Yin et al. 1988). Additional studies showed that lesions to or inactivation of opioid receptors in the Arc abolishes EA analgesia (Wang et al. 1990). Conclusively, opioid-mediated stimulation at the level of the brain and brainstem plays a pivotal role in activating mechanisms of acupuncture analgesia, like e.g. descending inhibition through the hypothalamus (Arc) – PAG – NRM - axis.

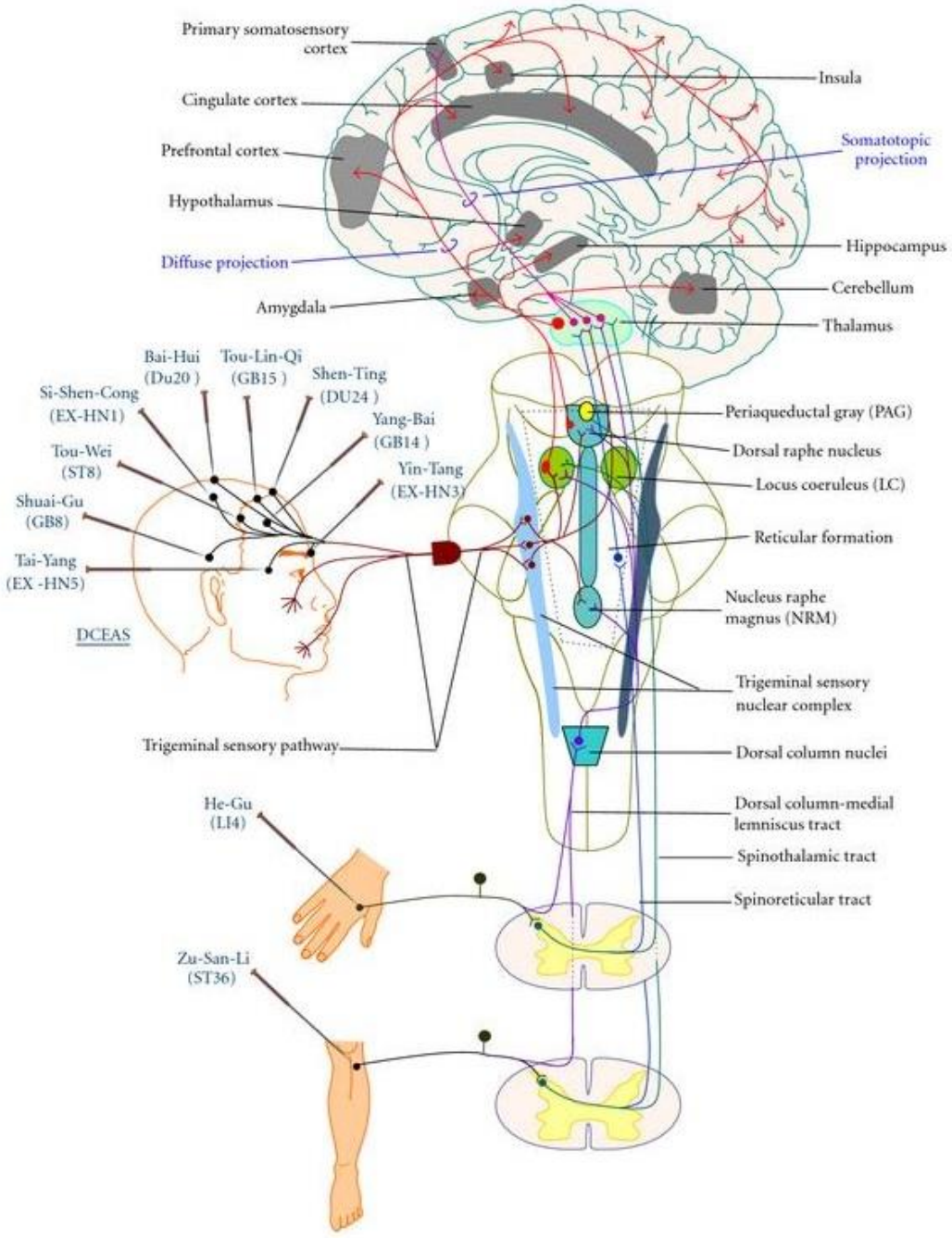

Figure 6. Schematic of the multiple afferent pathways facilitating the acupuncture signal from different peripheral sites to the brain. Grey shadows in brain are areas commonly seen activated following acupuncture stimulation in neuroimaging studies. DCEAS = dense cranial electroacupuncture stimulation. From (Zhang et al. 2012).

Acupuncture also exerts its analgesic effects through activation of the monoaminergic descending pain pathway (Murotani et al. 2010, Silva et al. 2011). The primary neuromodulators of this pathway, serotonin and noradrenaline, show an increase in concentration following acupuncture stimulation, as does the activation of serotonergic

receptors (Sprott et al. 1998, Yoshimoto et al. 2006, Zhang et al. 2011b). Serotonin has been shown to have analgesic properties in this descending system (Sprott et al. 1998), and noradrenaline has been proposed as an inhibitor of inflammatory pain (Zhang et al. 2012). Induction of this pathway produces analgesia, which can be dramatically reduced by serotonin receptor antagonists, proposing a major role for this pathway in acupuncture-induced analgesia (Sprott et al. 1998, Chang et al. 2004, Fry et al. 2014).

The descending inhibitory system consists of multiple nuclei and areas in the brain, such as NRM, PAG, LC and Arc, and it has been concluded that this system indeed plays a pivotal role in the generation of acupuncture analgesia (Zhao 2008). Studies have shown that injuries to the dorsal lateral funiculus of the spinal cord- where the inhibitory system descends- decreases or abolishes acupuncture analgesia (Hu et al. 1980, Li et al. 2007). As we know, EA activates the NRM (Liu et al. 1986, Liu 1996), but a lesion to the NRM or the dorsal lateral funiculus (DLF) decreases the effect from acupuncture significantly, even though in the DLF-lesion NRM neurons still are activated, suggesting a role for NRM in activating the descending inhibition system (Du and Chao 1976, Liu et al. 1986). Serotonin and its receptors are abundant in the NRM and EA is known to increase concentrations of serotonin and its metabolic products, especially in the NRM and in the spinal cord (Han et al. 1979a, Chang et al. 2004). Selective ablation of serotonin in the brain reduced EA analgesia markedly, whereas the use of a serotonin receptor antagonists almost fully eliminated the EA induced analgesia, hinting towards how important this system is for acupuncture analgesia (Han et al. 1979a, Chang et al. 2004).

2.3.4 Miscellaneous

Several other transmitters and bioactive compounds than those mentioned above have also been the target of extensive research. One that has gathered much interest is the cholecystokinin octapeptide (CCK-8), which is extensively distributed in different parts of the CNS and exerts anti-opioidergic activity through activation of the CCK-receptor, hence belonging to the pro-nociceptive phalanx of the pain processing system (Itoh et al. 1982, Watkins et al. 1985, Han 1995). An early study regarding acupuncture analgesia concluded that so-called non-responder rats, i.e. rats that only had minor analgesic effect as a result after EA, had a prominent rise in spinal CCK-release whereas responder rats, which had good analgesic effect following EA only had a slight increase in the spinal release of CCK

(Zhou et al. 1993). Later research in rats intriguingly showed that intracerebroventricular (i.c.v) administration of antisense oligonucleotides to CCK mRNA decreased both CCK mRNA and CCK-8 concentrations in the brain thereby turning non-responders into responders and potentiating the analgesic effect for EA analgesia and morphine, an opioid receptor agonist (Tang et al. 1997). Subsequent research on the topic has found that in non-responder rats, CCK receptor mRNA increases following high-frequency EA and that intrathecal injection of CCK-8 and CCK receptor antagonists reduces and potentiates EA analgesia, respectively (Ko et al. 2006, Huang et al. 2007). Due to the findings above it has been proposed that CCK release and CCK receptor density in the brain would, at least partly, count for individual differences in acupuncture analgesia.

In conclusion, the main networks propagating acupuncture analgesia is the opioid-mediated system and the descending inhibitory system mediated mainly by serotonin and noradrenaline acting towards anti-nociception in the CNS, while CCK exerts its pro-nociceptive effects on the opioid-mediated system in the CNS. Deactivation of the limbic system decreases the emotional input to the sensation of pain.

3 MATERIALS AND METHODS

3.1 Material

The material for the present, retrospective study was collected ad hoc during the years 2007-2014 at the Acupuncture Clinic of the Veterinary Teaching Hospital of University of Helsinki (I myself was not involved in the gathering of data, I simply explored and analysed it). The owners of the animals brought to the clinic, who agreed to participate, filled out questionnaires in Finnish before the first visit (Appendix I), to get a sense of how the animal was doing before starting treatment, and subsequently prior to some of their follow-up visits (Appendix II). The questionnaire included questions related to mobility and quality of life as well as use of pain medications, supplements, other treatments etc. Animals with a wide variety of disease were brought to the clinic, but included in this study were only dogs suffering from chronic musculoskeletal disease and/or chronic pain affecting the locomotor apparatus. When exploring the data from the questionnaires, inconsistencies in the way the owners had filled out the questionnaires as well as missing data for some dogs were discovered. While some animals had actual diagnoses (e.g.

osteoarthritis, disc-related disease, spondylosis) as reported by the owner, others were simply reported by the owner as suffering from e.g. ”chronic pain” or ”stiffness and limping” for a longer period of time. Inconsistencies in the completion of questionnaires and subsequent missing data constitute a severe limitation of the possibilities for exploring data. Therefore, we decided to focus this study on exploring a possible reduction in chronic pain as measured by the Helsinki Chronic Pain Index (HCPI) (Hielm-Björkman et al. 2009) and visual analogue scales (VAS) measuring mobility and quality of life for those cases possessing the sufficient data. Additionally, we took the last follow-up visits for each patient and explored those as well, so that the owner would have had as much time as possible to notice a possible effect of acupuncture treatment. From the 279 answers collected, only 118 could ultimately be used in this study. 23 answers were discarded due to the patient only having a 1st visit answer, 16 were discarded due to wrong diagnosis, 27 answers were taken out because of wrong or incomplete filling of the questionnaire and 3 were discarded because it was the wrong species (cat) and the remaining 92 answers we discarded were due to the patient’s owner answering multiple follow-up questionnaires, the use of which would have led to skewed results.

The HCPI (Appendix I on pages 1, 2 and 3, Appendix II on pages 2, 3 and 4, questions 1, 3, 4, 5, 7, 10, 11, 14, 15, 17 and 19, in Finnish) is a validated index to measure response to treatment, i.e. reduction or increase in pain levels in dogs suffering from chronic pain due to osteoarthritis (Hielm-Björkman et al. 2009). Even though all of the dogs in this study did not suffer from osteoarthritis, it was still used as a measurement for chronic pain. The HCPI consists of 11 questions, where the owner is to assess the status of the dog’s “mood”, “playfulness”, “walking”, “trotting”, “galloping”, “getting up”, “lying down”, “jumping”, “mobility after exercise”, “mobility after exercise + rest” and “vocalizing (pain)”. The owner is to check only 1 alternative out of 5 alternatives/question. The alternatives are one very positive, one positive, one quite neutral, one negative and one very negative and are scored 0-4; 0 for the most positive alternative and 4 for the most negative, giving the sum of the answers an index that ranges between 0-44, where dogs with scoring under 6 are considered pain-free and dogs with scoring above 12 are considered to suffer from chronic pain. Dogs with scores 6-11, however, make up for a grey zone and might or might not be painful (Hielm-Björkman et al. 2009). The VASs (Appendix I, page 3 and Appendix II, page 4) were measured for mobility (actual wording in Finnish: difficulties in mobility) and quality of life on a 100 mm (=10cm) line, such that the owner would mark an X on the

point of the line describing the dog's status in relation to the best/worst status possible. The distance from the left end of the line to the X was measured with a ruler in each case and the answer coded in millimetres such that 0 mm represented the best scenario while 100 mm represented the worst.

On page 5 of the follow-up questionnaire (Appendix II, "Vertaileva kysely"; freely translated as comparative enquiry), the owners were invited to answer a set of questions involving mobility, pain, quality of life and skin/fur condition. The owners were asked to compare the present state to that before starting acupuncture treatment, and to assess if the dog's condition in respect to the question was "much better", "slightly better", "unchanged", "slightly worse" or "much worse". Only one alternative per question could be checked. Due to focus on locomotor and pain problems, the answers about skin/fur condition were left out of this study.

3.2 Statistical analysis

Statistical analysis for this study was carried out using SPSS (IBM™ SPSS™ Statistics V. 23.0). Descriptives were calculated for the average and differences between first and latter visits. As the data was normally distributed, independent samples *t*-test was used for inter-group analysis in the comparative enquiry part, while paired samples *t*-test was used for analysis in the HCPI –and VAS-groups. $P < 0.05$ was deemed as a statistically significant difference.

4 RESULTS

The cases with data to sum up a HCPI-score for first and subsequent visits, made it possible to study four different groups (see tables 1 and 2 for results). In the first group (HCPI-group 1), the baseline-HCPI and –VAS were compared to those of the second visit. Number of cases in this group were 7, of whom 3 were males (42.9%) and 4 females (57.1%). The ages of the dogs ranged between 3 and 10, with a mean of 6.36. Four of the dogs (57.1%) had owner-reported osteoarthritis (OA), while 3 of the dogs (42.9%) suffered from chronic pain in general. 3 of the dogs received non-steroidal anti-inflammatory drugs

(NSAIDs) (2 daily, 1 sporadically), while 3 dogs received no pain medication and 1 dog received both NSAIDs and gabapentin daily at the first visit. On the second visit, 4 of the dogs did not receive any pain medication, while 2 got NSAIDs (1 daily, 1 sporadically) and 1 dog received NSAIDs + gabapentin regularly. Supplements were given to all dogs in this group. For all dogs, the interval between the first and second visit was 7 days.

The second group (HCPI-group 2) had their baseline-HCPI and VAS-scores compared to the third-visit scores. Cases in this group amounted to 9, with 5 males (55.6%) and 4 females (44.4%) and an age-range of 0.75-13, the mean being 6.92. 4 dogs (44.4%) were suffering from OA while the rest (5 cases, 55.6%) suffered from diffuse chronic pain and/or weakness/stiffness in general. Out of these cases, 4 did not receive any pain medication, while 2 were receiving NSAIDs when necessary, 2 were getting NSAIDs and gabapentin and one was receiving tramadol at the first visit. At the later visit 5 dogs did not receive any pain medication, while 1 was getting NSAIDs, 1 tramadol and 2 NSAIDs + gabapentin on a daily basis. All dogs in the group except for one received supplements regularly. The number of days between the first and the third visit ranged between 13 and 49, the mean being 18.89 days and the median being 14 days.

HCPI-group 3 contained 5 cases, for whom the baseline-HCPI and –VAS were measured against those of the fourth visit. Two cases were male and 3 female (60%). Two were suffering from OA while the remaining 3 cases had diffuse chronic pain as reported by the owner. Ages in this group ranged from 3 to 13 with a mean of 6.90. 2 cases received NSAIDs, one tramadol and 2 did not receive any pain medication at the first visit, while at the later visit only 1 dog received pain medication (NSAIDs) and the rest did not receive any. Four dogs were given supplements at the first visit, while one dog did not get any. At the later visit, only two of the dogs still were given supplements and three of them were not. Number of days between the two visits were between 20 and 57, with a median of 31 and a mean of 34.2 days.

The fourth HCPI-group (HCPI-group 4) had 7 cases, of whom 3 were male and 4 female. In this group the values of the first visit were compared to the values at a visit 3-6 months later, with a range of 105-174 days between visits, the mean being 127 days. The dogs were between 3 and 14 years of age, with a mean of 8.36 years. Four dogs suffered from OA while 3 dogs suffered from owner-reported diffuse chronic pain. Two of the dogs did not receive any pain medication at the first visit, 3 dogs got pain medication sporadically (2 got NSAIDs, 1 got tramadol) and the remaining 2 got pain medication daily (1 got

tramadol, 1 got gabapentin). At the later visit 5 dogs did not receive pain medication at all, whereas 1 still got it sporadically (NSAIDs) and 1 daily (gabapentin).

Analysing the HCPI and VAS values for the different visits within groups one can see that the mean of both HCPI-scores as well as VAS-scores have decreased. However, no statistically significant difference could be found for any of the values or between any of the groups.

Table 1. Means and standard deviations for HCPI- and VAS-scores, comparing the first visit with subsequent ones. HCPI= Helsinki chronic pain index. SD= standard deviation. M-VAS= mobility VAS. Q-VAS= quality of life VAS

		HCPI		M-VAS		Q-VAS	
		Mean	SD	Mean	SD	Mean	SD
Group 1	1st visit	12.71	4.57	41.29	24.34	34.14	21.29
	2nd visit	9.86	4.10	36.71	24.79	22.71	18.23
Group 2	1st visit	12.33	7.57	41.22	21.79	32.67	19.92
	3rd visit	11.89	5.78	34.78	20.49	27.56	14.80
Group 3	1st visit	14.60	9.24	40.60	24.47	28.80	25.00
	4th visit	11.20	4.32	34.60	22.77	24.60	17.14
Group 4	1st visit	14.00	7.72	45.71	25.72	32.86	20.33
	Later visit	11.00	4.83	25.29	18.08	29.43	14.95

Table 2. P-values from comparing first and later visits for the different groups. P < 0.05 = significant difference. HCPI= Helsinki chronic pain index. M-VAS= mobility VAS. Q-VAS= quality of life VAS.

	P(HCPI)	P(M-VAS)	P(Q-VAS)
Group 1	0.091	0.642	0.082
Group 2	0.731	0.110	0.359
Group 3	0.362	0.545	0.580
Group 4	0.242	0.119	0.722

In the final part of this study, since the number of cases in the HCPI-groups were so small, we gathered together as many data-rich cases of the comparative enquiry as possible, and after selecting all the last available visits of follow-up patients with an owner-reported chronic pain diagnosis (e.g. osteoarthritis) or owner-reported chronic pain symptoms affecting the locomotor apparatus, 85 eligible cases remained, none of which were completely filled out questionnaires. Thus, in the analyses presented below, the number of cases (answers) varies from question to question. The group of 85 dogs consisted of 46

(54.1%) females and 39 males (45.9%). Ages of the dogs ranged from 2-16 years, with a mean of 7.95 and median of 7 years. Owners reported 39 dogs (45.9%) suffering from osteoarthritis, 8 (9.4%) from spondylosis, 11 (12.9%) from pain/symptoms related to intervertebral discs (e.g. disc protrusion) and 27 (31.8%) dogs suffering from chronic pain and/or stiffness/weakness in general, with no reported definitive diagnosis. Supplements, such as glucosamines and omega fatty acids, were regularly given to 58 of the dogs (68.2%), while 27 dogs (31.8%) did not receive any. The results from the basic statistical analysis of the material available are shown in table 3.

Table 3. Frequencies of answers to the different questions, given as number of cases, with the percentage related to all answers in captions. Number of answers to the particular question in captions after question.

Question	Much better	Slightly better	Unchanged	Slightly worse	Much worse
Mobility (n=85)	25 *(29.4%)*	29 *(34.1%)*	22 *(25.9%)*	6 *(7.1%)*	3 *(3.5%)*
Walking up stairs (n=82)	14 *(17.1%)*	15 *(18.3%)*	51 *(62.2%)*	1 *(1.2%)*	1 *(1.2%)*
Walking down stairs (n=81)	11 *(13.6%)*	13 *(16.0%)*	53 *(65.4%)*	4 *(4.9%)*	0 *(0 %)*
Lying down (n=83)	9 *(10.8%)*	18 *(21.7%)*	53 *(63.9%)*	2 *(2.4%)*	1 *(1.2%)*
Getting up (n=84)	10 *(11.9%)*	17 *(20.2%)*	52 *(61.9%)*	4 *(4.8%)*	1 *(1.2%)*
Climbing (n=81)	9 *(11.1%)*	18 *(22.2%)*	52 *(64.2%)*	2 *(2.5%)*	0 *(0 %)*
Jumping (n=81)	11 *(13.6%)*	23 *(28.4%)*	40 *(49.4%)*	7 *(8.6%)*	0 *(0 %)*
Walking (n=85)	12 *(14.1%)*	31 *(36.5%)*	35 *(41.2%)*	6 *(7.1%)*	1 *(1.2%)*
Trotting (n=82)	10 *(12.2%)*	26 *(31.7%)*	40 *(48.8%)*	6 *(7.3%)*	0 *(0 %)*
Galloping (n=80)	5 *(6.3%)*	21 *(26.3%)*	50 *(62.5%)*	3 *(3.8%)*	1 *(1.3%)*
Pacing (n=70)	4 *(5.7%)*	11 *(15.7%)*	53 *(75.7%)*	1 *(1.4%)*	1 *(1.4%)*
"Bunny jumping" (n=70)	6 *(8.6%)*	14 *(20.0%)*	47 *(67.1%)*	1 *(1.4%)*	2 *(2.9%)*
Moving on own initiative (n=83)	14 *(16.9%)*	20 *(24.1%)*	42 *(50.6%)*	7 *(8.4%)*	0 *(0 %)*
Mobility after rest (n=79)	11 *(13.9%)*	24 *(30.4%)*	41 *(51.9%)*	3 *(3.8%)*	0 *(0 %)*
Mobility after exercise (n=75)	10 *(13.3%)*	20 *(26.7%)*	41 *(54.7%)*	2 *(2.7%)*	2 *(2.7%)*
Mobility after exercise + rest (n=84)	14 *(17.3%)*	22 *(27.2%)*	42 *(51.9%)*	2 *(2.5%)*	1 *(1.2%)*
Pain- overall (n=78)	24 *(30.8%)*	31 *(39.7%)*	18 *(23.1%)*	4 *(5.1%)*	1 *(1.3%)*
Pain stretching hindlegs (n=66)	8 *(12.1%)*	16 *(24.2%)*	38 *(57.6%)*	3 *(4.5%)*	1 *(1.5%)*
Panting (n=73)	9 *(12.3%)*	15 *(20.5%)*	46 *(63.0%)*	2 *(2.7%)*	1 *(1.4%)*
Vocalizing (pain) (n=68)	9 *(13.2%)*	11 *(16.2%)*	46 *(67.6%)*	1 *(1.5%)*	1 *(1.5%)*
Mood (n=85)	23 *(27.1%)*	34 *(40.0%)*	22 *(25.9%)*	6 *(7.1%)*	0 *(0 %)*
Sociability (n=84)	16 *(19.0%)*	27 *(32.1%)*	37 *(44.0%)*	4 *(4.8%)*	0 *(0 %)*
Playfulness (n=83)	20 *(24.1%)*	30 *(36.1%)*	29 *(34.9%)*	4 *(4.8%)*	0 *(0 %)*
Quality of life (n=85)	26 *(30.6%)*	39 *(45.9%)*	15 *(17.6%)*	4 *(4.7%)*	1 *(1.2%)*

When asked about pain medication recently, 22 owners (25.9%) reported the dog getting pain medication daily or almost daily during the last month. Of these dogs 8 got some kind of NSAID, 9 got gabapentin, 1 got opioids (tramadol), 1 got corticosteroids and 2 got a combination of NSAIDs and gabapentin. 46 out of 85 (54.1%) dogs had not gotten any pain medication at all recently, while the remaining 17 dogs (20.0%) had gotten pain medication sporadically over the last month.

The material from the comparative enquiry was further analysed by grouping the dogs according to different criteria to detect possible differences between the groups. Firstly, the dogs were grouped by diagnosis. Since definitive diagnoses were not available from the material, the dogs were simply divided into an osteoarthritis group (OA-group, N=39) and other chronic pain group (OCP-group, N=46). No statistically significant difference was found between the two groups ($p > 0.329$ for all questions).

Secondly, the dogs were grouped by whether or not they had received pain medication. As noted above, 46 dogs had not gotten any pain medication recently, while 39 dogs had received some (22 dogs often and 17 sporadically). A significant difference was found only for the “galloping” question ($p=0.019$), where the non-medicated group had a lower mean, i.e. possible improvement compared to the medicated group. No other statistically significant differences were found between the two groups.

Lastly, two groups were created and compared on the basis of whether the dog had received supplements or not. While 27 dogs had not received any supplements, the remaining 58 dogs got some kind of supplement. Significant differences occurred for the items “galloping” ($p=0.010$), “bunny jumping” ($p=0.026$), “pain- overall” ($p=0.045$) and “vocalizing (pain)” ($p=0.022$). In each of these questions the mean was lower (i.e. on the “better” side) in the non-supplement group. No other statistically significant differences were detected between the two groups.

5 DISCUSSION

5.1 Current study

Granted, due to the nature of the study reported above, the statistical significance for the analyses carried out are not very convincing and thus, far-reaching conclusions can hardly be drawn. However, the results of the study may be interpreted as indicative for the efficacy of acupuncture in the treatment of chronic pain. No statistically significant differences were noted in the HCPI-groups, but the baseline levels of all four HCPI groups had means >12 that indicate chronic pain (12.33-14.60), whereas all means were lower than the pain threshold of 12 (9.89-11.89) after the acupuncture treatments (scoring of 6-11 might or might not be painful, as noted above). Also, the indicators measured using visual analogue scales were consequently better at the later visits, some with quite big differences (e.g. Q-VAS in group 1; 34.14 at first visit and 22.71 at second visit or M-VAS in group 4; 45.71 at first visit and 25.29 at latter). In addition to that, pain medication was reduced in all HCPI-groups when comparing 1st and subsequent visits. However, the owners coming to the clinic are asked not to reduce the pain medication during the first 3 visits, unless the dogs are showing adverse signs to them (e.g. gastric irritation/ulcer, liver or kidney-failure), so the reduction in medication in HCPI-groups 1 and 2 could be due to adverse reactions. Taken together, the results could indicate an improvement in pain levels in these dogs. The low number of cases in each group probably played a part in the statistically insignificant results, and with a greater number of cases, the results might have been different, i.e. shown a statistically significant difference.

Looking at the results of the comparative enquiry, although around 50-60% of the owners answered “unchanged” to most questions, the great majority of the remaining cases were put on the “better” side, with only sporadic cases ending up on the “worse” side. One must take into consideration though, that an answer of “unchanged” is hard to interpret, since we do not know if the dog actually has had difficulties with the particular question/problem based on the comparative enquiry, and thus “unchanged” might be bad if the dog really has a problem, but good if there was no problem/difficulty to begin with. A few questions particularly stood out in a positive manner, as “quality of life”, “pain- overall”, “mood”, “mobility” and “playfulness” gained 76.5%, 70.5%, 67.1%, 63.5% and 60.1% on the “better” side, respectively. The positive results to these questions, some of which do not assess a particularly specific problem, but rather maybe reflect health and well-being in a broader sense in a dog, again, could be indicative of acupuncture relieving chronic pain and thus affecting the overall well-being of the dog. This is in accordance with a previous

study where dog owners were not able to assess increased or decreased pain in their dogs as they did not recognize the symptoms associated with chronic pain; they could, however, assess symptoms such as quality of life, mobility, mood etc. (Hielm-Björkman et al. 2003, Hielm-Björkman et al. 2011)

Assessing the questions to which statistical differences were found; a significant difference to the "galloping" question when dividing the dogs into pain medicated or non-medicated groups could just be a coincidence, since no significant statistical difference was found to the other questions. As for the four questions for which a difference was found when comparing supplement vs no supplement groups, the size of the groups was quite uneven (58 vs 27). Considering how many had answered the particular questions- "galloping" (54 vs 26), "bunny jumping" (51 vs 19), pain -overall (55 vs 23) and "vocalizing (pain) (50 vs 18) - and considering the fact that around 50-60% answered "unchanged" to most questions, it is not inconceivable to think that a few "better" answers in the smaller group would account for a significant difference.

It seems, based on the results of this study that the mobility, painfulness and quality of life improved in many of the dogs included. As these variables all are indicative of pain, they indicate a positive effect after acupuncture. However, a multitude of factors could have impacted the results seen in this study. With great discrepancies in the answering of the questionnaires, the material at hand was not very good, especially in the process of statistically ruling out the effects of some other factors. Owners also reported a plethora of other treatments that had been used, including physiotherapy, osteopathy, chiropractic, homeopathic medicines, insertion of gold implants, swimming etc. In some cases the treatment had been implemented even years before the day of the filling of the questionnaire, but in most cases there was no mention of when the treatment had been applied. Many owners also reported giving their dogs a wide array of supplements, most commonly glucosamines, methylsulfonylmethane (MSM), chondroitin sulphate and omega fatty acids, but there was no mention of how long they had used these supplements.

In addition to these factors, there are several other ones that could interfere with the results. The owner could have simply forgotten what the baseline-mobility and –pain was when answering the comparative enquiry, and therefore thinks it has gotten better (or worse). As some of the dogs had been getting acupuncture for quite some time, even years, at the time of answering, assessing the baseline status at the first visit becomes an immensely challenging task. The owner might also be biased in the sense that he or she really

believes/hopes acupuncture is going to help the dog so he/she sees the dog's condition as being better than it actually might be. Another aspect of this could be a so called "secondary placebo effect", where the owner might have been feeling bad because of the dog's chronic pain or e.g. limping, and when treatment starts, the owner gets happier, which in turn could affect the mood of the dog for the better, thus making the dog's condition at least partly "improved" in the eyes of the owner. Awareness of the disease in question could also be a factor weighing in on the results in a positive manner, i.e. if the owner has gotten good information about e.g. osteoarthritis and how to prevent the progression of the disease, he/she might have made even major changes in the everyday life of the dog not mentioned in the questionnaire, which, again, might be seen as improvement. Lastly, the owner's ability to assess the pain level of the dog influences the results in studies like this. Even though the owners probably know the ways their dog behaves, moves and reacts to pain etc., chronic pain still is challenging to recognize if the owners do not know what they are looking for.

Evaluating the results of this current study in the light of all the factors that might be playing in, these results can be interpreted as indicative at best in aspect of reduction in chronic pain levels and improvement of mobility and quality of life solely due to acupuncture. The questionnaires used in this study seem straightforward to me and the wording of the questions leaves little or no room for misinterpretations by the person filling out the questionnaire, in this case the owner. The questionnaires used in the study at hand could in my opinion well be used in future studies, since the missing data and inconsistencies were not due the questionnaires themselves. However, instead of having open answers to questions about e.g. supplements and (pain) medication, these questions could also be carried out as multiple choice questions. This could make it easier to group the cases. A future study based on these questionnaires would require better planning for the study though, to make sure that the answers to first and subsequent visits are filled out properly and to as great an extent as possible.

5.2 Acupuncture research

Acupuncture analgesic mechanisms and the efficacy thereof have been subject to extensive research, especially during the last few decades. However, neither conclusive proof nor disproof have been found, owing partially to the difficulty of setting up a reliable randomized, controlled study (Deng et al. 2015). A particular problem arises from the fact

that studies with a non-acupuncture control group often show positive results regarding acupuncture analgesia, while studies with a sham-acupuncture control group tend to show that there is not a significant difference between the two groups (Itoh et al. 2008). The task of creating and standardizing a working and reliable form of sham acupuncture has proven more elusive than expected, and different methods have been tried during recent years, including acupuncture at a non-meridian, non-acupoint, shallow insertion of the needle and "placebo-needles", where the needle doesn't penetrate the skin but only pricks it and the needle retracts up into the handle (Langevin et al. 2006). It has been shown, though, that invasive as well as non-invasive sham acupuncture produces biophysical responses at the place of stimulation as well as activation in some of the same brain regions as in pain modulation (Pariente et al. 2005, Langevin et al. 2006). The analgesic effect (in humans) resulting from acupuncture treatment has been suspected to contain a moderate if not large amount of placebo-effect, owing to e.g. expectancy and belief in the treatment (Vickers et al. 2012). The placebo-effect exerted by sham acupuncture has been shown to be greater than that of a placebo-pill (Kaptchuk et al. 2006). Also, neuroimaging studies have shown that the brain areas activated by placebo differ from the pain-modulating brain areas activated by an acupuncture-specific effect, the specific mechanism of which is yet to be fully elucidated, but include e.g. short and long-term changes in μ-opioid receptor activity in certain parts of the brain (Harris et al. 2009, Kong et al. 2009a, Kong et al. 2009b). Since verum acupuncture and sham acupuncture both seem to induce analgesia, though sometimes at different intensities, one theory is that sham acupuncture would elicit the same response as real acupuncture, but to a lesser extent (Hammerschlag and Zwickey 2006).

The treatment protocol, i.e. the way and how often acupuncture treatment is given, has also shown to be of importance. As mentioned above, EA-stimulation at different frequencies elicits the release of endogenous opioids at different ratios (Fei et al. 1987, He and Han 1990, Han et al. 1999). A neuroimaging study showed that treatment durations of various lengths impacted the effects in the brain in different ways (Li et al. 2006). Of two studies (Macklin et al. 2006, Flachskampf et al. 2007), both treating hypertension with acupuncture, one came to the conclusion that acupuncture was no better in treating hypertension than was sham acupuncture, while the other study reported a significant difference between the two groups. The treatment protocols were different for the two studies, especially in the frequency at which acupuncture was given; the study which

concluded a significant difference present when comparing verum and sham acupuncture, gave acupuncture treatment more often and more times altogether than did the researchers in the other study, which could be indicative of "too little treatment/too low dose" in the study where there was no difference (Macklin et al. 2006, Flachskampf et al. 2007). Various needle-manipulation techniques during treatment and minor variances in these have been proven to give rise to different kinds and intensities of cellular responses at the site of stimulation (Langevin et al. 2007). This could mean that the efficacy of acupuncture is partly dose- and technique-dependent.

While the placebo-effect comprises a major challenge in human studies of acupuncture mechanisms and efficacy, the same can't be said for animal studies. However, it does not mean that acupuncture studies in animals are without challenges, such as the animal's possible response to the situation e.g. fear following restraint, differences in disease severity or even interpreting the results (measuring pain). Whereas acupuncture studies with sham acupuncture groups as control usually can find no difference between the real and the sham acupuncture groups, studies done on animals in the same manner tend to show a positive difference for acupuncture compared to the controls (Langevin et al. 2006). I do not see it as inconceivable, though, that a dog receiving multiple acupuncture treatments which have relieved pain, could develop a placebo effect due to positive expectancy as it comes to the clinic for its next treatment. This could further complicate the acupuncture research in animals and should be taken into consideration.

While it has been difficult to show that an acupoint gives rise to a certain response in the body different to that of a non-acupoint, several animal models have shown variations therein, strengthening the theory of acupoint specificity as being a relevant factor for the efficacy of treatment (Lee and Beitz 1993, Koo et al. 2002, Lao et al. 2004, Tjen-A-Looi et al. 2004, Zhao et al. 2006). Some factors might, however, be attributable to or at least play a role in these results; animals used in these types of studies have never before been exposed to acupuncture treatment; the disease severity as well as type of pathology might be highly controllable to fit the study, e.g. injection of specific amounts of inflammatory agents; animals used for experiments are usually genetically homogenous, showing little differences between individuals and thus diminishing the standard deviation in the statistical analyses; as the purpose with animal experiments often is to elucidate a mechanism of action, animals that have failed to elicit a desired response might be underreported altogether (Langevin et al. 2006).

As mentioned earlier, illness in TCM-theory is due to an imbalance of Yin and Yang and disruption of Qi-flow in the body, i.e. the homeostasis is disturbed. The disturbance in Qi-flow sensitizes acupoints along the particular meridian where the disturbance occurs. Since the meaning of acupuncture is to restore homeostasis in the body, could it mean that the more severe the disease gets, i.e. further away from homeostasis, the better acupuncture actually works? In that case differences should easily be seen in studies with non-acupuncture controls, as also seems to be the case (Deng et al. 2015). This theory together with the theory of any point on the body eliciting a response, only to a lesser extent than a real acupoint (Hammerschlag and Zwickey 2006), could further partially explain why the difference between real and sham acupuncture usually is not found in studies; if the whole body is sensitized, even sham acupuncture or non-acupuncture could give rise to a reaction strong enough to diminish the statistical difference between verum acupuncture and control. Even though it seems clear that the placebo effect has a major impact on the outcome of treatment, one should keep in mind that it might not account for all the cases where the effects of real and sham acupuncture have been declared statistically equivalent.

Much like with pharmaceuticals, there are clear individual as well as gender differences in the responsiveness to acupuncture treatment (Lund and Lundeberg 2010). Sources report a non-responsiveness between 10% and 30% for humans and animals (Han 1994, Langevin et al. 2006), which definitely could account for some of the discrepancies and downright contradictory results appearing in studies during recent years. If a person (or animal) doesn't respond to treatment, it could mean that the dosage might be too small, i.e. the treatment not efficient enough to resolve the problem, and, thus, more treatment is required e.g. as longer sessions or a higher frequency of treatments. Another possible factor that could be interfering with the results might be "wrong" treatment according to TCM, assuming that acupoint specificity is essential for the treatment. Say that a person is being treated for a headache (symptom), the treatment according to TCM-teachings should normalize the Qi-flow and regain homeostasis of the body. However, the result of the disturbance of Qi-flow (illness) has led to the symptom of headache, but many different disturbances in Qi-flow can probably lead to the symptom of headache, e.g. stagnation of Qi in the liver meridian or too little Qi in the kidney meridian. The selection of acupoints in TCM is based on where the diagnosed disturbance in Qi-flow is, i.e. if the Qi-flow in the liver meridian is abnormal, treatment/stimulation of the kidney meridian might not help. Rather than acupuncture being a work/does not work treatment, it is becoming clearer that,

again, acupuncture treatment might resemble pharmaceutical treatment more than expected, i.e. right medicine at the right dose is needed to treat a certain illness. This way, maybe if we can encode the exact mechanism of action for acupuncture that could lead to specific acupuncture treatments. However, it could also be the case that acupuncture has different modes of action depending on the disease in question, e.g. the responses occurring in the body following acupuncture treatment for hypertension could be different than the responses following treatment of chronic pain. As the point of acupuncture treatment is to regain homeostasis, it seems strange to think that normalizing blood-pressure from either hyper- or hypotension could be the work of the same exact mechanism. If the mechanisms really are different, it is essential for future studies to look at the pathologic process in question when assessing the efficacy of treatment to understand if it works, why it works and how it works.

A quite recent study proposed a new anatomical-physiological model for how acupuncture analgesia works (Silberstein 2009). In this model, a cutaneous, pain-transmitting C-fibre afferent with its soma in the dorsal root ganglion rises to a subepidermal spot, where it bifurcates in a T-shaped manner with the branches going parallel to the skin. Merkel cells in the skin are known to be intimately associated with C-fibre nerve endings (Zhang et al. 2002a) and Merkel cells possess the ability of neurotransmitter release (Haeberle et al. 2004). This way, the firing of a Merkel cell would lead to signal transmission in two directions; to the cell soma of the neuron and to the next Merkel cell. Silberstein suggests a neural network continuously transmitting excitatory impulses at some distance from the terminals of local nociceptors. This ongoing firing of impulses stimulating local nociceptors, in this case C-fibres, would be balanced by continuous firing of Aβ-fibres. An acupuncture needle put into the bifurcation of the afferent C-fibre axon would thus disrupt the signals going through it (nociception) and therefore lead to pain relief. If, correct, this theory would give rise to a fourth dimension of the autonomic nervous system (ANS), the first three being the sympathetic, parasympathetic and enteric nervous system. The author used the term visceral afferent nervous system to describe the model presented above (Silberstein 2009).

Acupuncture has proven an elusive adversary in the quest for science to totally understand it. It is becoming clearer all the time that acupuncture research in the future has to change its tactics, since the outcome thus far has been inconclusive and even contradictory. This, however, is not exclusive for acupuncture research; complete mechanisms of actions

remains to be elucidated for commonly used NSAIDS (Lees et al. 2004) as well as neuropathic pain medications such as gabapentin (Kukkar et al. 2013). Since animal and human studies both have their own strengths and challenges, combining the results of the two groups perhaps could elucidate the mystery of acupuncture further. This, of course, assuming that researchers play to the strengths of human and animal studies respectively, e.g. studying mechanisms of action in animals and when that is clear, determining efficacy in studies conducted with humans. Standardization of trials would also be valuable, but might be hard to execute at the moment, as it would first need a clearer mapping of all already known working mechanisms and then a hypothesis of understanding the mechanism of acupuncture; is it really a certain, similar response each time or is it indeed a disease-specific response?

6 ABBREVIATION INDEX

5-HT = serotonin
Ac = nucleus accumbens
ACC = anterior cingular cortex
Amyg = amygdala
AMPA receptor = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ANS = autonomic nervous system
Arc = arcuate nucleus
ATP = adenosine triphosphate
BG = basal ganglia
CCK-8 = cholecystokinin octapeptide
Cd = caudate nucleus
CGRP = calcitonin gene-related peptide
CM = centromedian nucleus
CNS = central nervous system
CSF = cerebrospinal fluid
CVLM = caudal ventrolateral medulla
DLF = dorsolateral funiculus
DRG = dorsal root ganglion
EA = electroacupuncture
FAAH = fatty acid amid hydrolase
FRAP = fluoride-resistant acid phosphatase
GABA = γ-aminobutyric acid
GPCR = G-protein coupled receptor
Hab = habenular nucleus
HCPI = Helsinki chronic pain index
HT = hypothalamus
IB4 = isolectin B4
IC = insular cortex
i.c.v. = intracereborventricular
IL = interleukin
LC = locus coeruleus
LPB = lateral parabrachial area
mGluR = metabotropic glutamate receptor
MIA = mechanically insensitive afferents
MSA = mechanically sensitive afferents
MSM = methylsulfonylmethane
MTrP = myofascial trigger point
M1 = primary motor cortex
nACh R = nicotinic acetylcholine receptor
NGF = nerve growth factor
NKA/NKK = neurokinin A/K
NK1R = neurokinin 1 receptor
NMDA receptor = N-methyl-D-aspartate receptor
NO = nitrous oxide
NRM = nucleus raphe magnus
NSAIDs = non-steroidal anti-inflammatory drugs
NTS = nucleus of the solitary tract
OA = osteoarthritis

PAG = periaqueductal grey

PB = parabrachial nucleus

PCC = posterior cingulate cortex

PFC = prefrontal cortex

PO = preoptic area

PVH = hypothalamic paraventricular nucleus

RVM = rostroventral medulla

Sm = nucleus submedius

SMA = supplementary motor cortex

SP = substance P

SST = somatostatin

STT = spinothalamic tract

S1 = primary somatosensory cortex

S2 = secondary somatosensory cortex

TCM = traditional Chinese medicine

Th = thalamus

TNF-α = tumour necrosis factor α

TRPV1 = transient receptor potential vanilloid 1

VAS = visual analogue scale

WBCs = white blood cells

WDRs = wide dynamic range neurons

VLF = ventrolateral funiculus

WM = western medicine

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Appendix I
AKUPUNKTIOKLINIKAN ALKUKAAVAKE

Päiväys_____ Koiran nimi _____ Ikä _____ Sukup. ____

Koiran diagnoosi/ mistä hoidetaan? _____

Koiran lääkitys: _____

Edelliset hoidot ja pvm: _____

Koiran pääoireet? _____

Omistajan Nimi_____

Kaavakkeen täyttäjän allekirjoitus_____

Muistakaa että:

- Jokaiseen kysymykseen yksi vastaus. (Ei nolla eikä kaksi)
- Aina vastaatte joka kysymykseen samalla lailla joka kerta (esim. koira ehkä hyppää sohvaan ja autoon eri lailla jolloin on tärkeätä että aina vastaatte kysymykseen ajatellen samaa tilannetta ja että vastaatte aina esim pitkän lenkin jälkeen eikä ennen, jolloin vertaileva tilanne aina on sama).
- Aina sama ihminen / samat ihmiset vastaavat kyselyyn joka kerta, jolloin vastaukset ovat vertailukelpoisia
- Merkatkaa miten olette käyttäneet kipulääkkeitä
- Aina ilmoitatte, jos koiranne vahingossa on syönyt jotakin muuta ruokaa, ravintovalmisteita yms.
- Aina ilmoitatte, jos koiranne vahingossa onkin saanut jotakin muuta hoitoa

Potilaan yleistila nyt

Rastita yksi vaihtoehto / kysymys; se joka parhaiten vastaa koirasi tilaa **menneellä viikolla**.

1. Mielentila on:

erittäin virkeä	virkeä	ei virkeä, eikä apea	apea	erittäin apea
□	□	□	□	□

2. Koira heiluttaa häntäänsä:

hyvin usein	usein	silloin tällöin	harvoin	hyvin harvoin
□	□	□	□	□

3. Koira leikkii:

hyvin mielellään	mielellään	vastahakoisesti	hyvin vastahakoisesti	ei ollenkaan
□	□	□	□	□

4. Koira kävelee:

hyvin mielellään	mielellään	ei mielellään, eikä vastahakoisesti	vastahakoisesti	hyvin vastahakoisesti
□	□	□	□	□

5. Koira ravaa (siirtää ristikkäistä etu- ja takajalkaa samanaikaisesti):

hyvin mielellään	mielellään	vastahakoisesti	hyvin vastahakoisesti	ei ollenkaan
□	□	□	□	□

6. Koira peitsaa (siirtää samanpuoleista etu- ja takajalkaa samanaikaisesti):

hyvin harvoin	harvoin	silloin tällöin	usein	hyvin usein
□	□	□	□	□

7. Koira laukkaa:

hyvin mielellään	mielellään	vastahakoisesti	hyvin vastahakoisesti	ei ollenkaan
□	□	□	□	□

8. Koiran tapa laukata muistuttaa takaa jänistä: molemmat takajalat liikkuvat yhdessä

hyvin harvoin	harvoin	silloin tällöin	usein	hyvin usein
□	□	□	□	□

9. Koira liikkuu oma-aloitteisesti ulkona:

hyvin mielellään	mielellään	vastahakoisesti	hyvin vastahakoisesti	ei ollenkaan
□	□	□	□	□

10. Koira liikkuu kovan rasituksen ja senjälkeisen levon jälkeen:

erittäin helposti	helposti	kohtalaisesti	vaikeasti	hyvin vaikeasti
□	□	□	□	□

11. Koira hyppää (esim. sohvaan, autoon tms.):

hyvin mielellään	mielellään	vastahakoisesti	hyvin vastahakoisesti	ei ollenkaan
□	□	□	□	□

12. Koira kulkee rappusia ylös:

hyvin mielellään	mielellään	vastahakoisesti	hyvin vastahakoisesti	ei ollenkaan
□	□	□	□	□

13. Koira kulkee rappusia alas:

hyvin mielellään	mielellään	vastahakoisesti	hyvin vastahakoisesti	ei ollenkaan
□	□	□	□	□

14. Koira menee makuulle:

erittäin helposti	helposti	kohtalaisesti	vaikeasti	hyvin vaikeasti
□	□	□	□	□

15. Koira nousee makuulta:

erittäin helposti	helposti	kohtalaisesti	vaikeasti	hyvin vaikeasti
□	□	□	□	□

16. Koira läähättää kivun vuoksi:

ei juuri koskaan	harvoin	joskus	usein	hyvin usein
□	□	□	□	□

17. Koira valittaa kipuja:

ei juuri koskaan	harvoin	joskus	usein	hyvin usein
□	□	□	□	□

18. Koira valittaa kun takajalkoja venytetään taakse:

ei juuri koskaan	harvoin	joskus	usein	hyvin usein
□	□	□	□	□

19. Koira liikkuu kovan rasituksen jälkeen:

erittäin helposti	helposti	kohtalaisesti	vaikeasti	hyvin vaikeasti
□	□	□	□	□

Koiran liikuntavaikeudet

Arvioi koiran liikkumavaikeus piirtämällä rasti alla olevalle janalle, siihen missä se parhaiten kuvaa tämänhetkisen tilanteen:

Ei mitään vaikeuksia _____ Pahin mahdollinen tilanne

Elämänlaatu

Arvioi koiran elämänlaatua piirtämällä rasti alla olevalle janalle, siihen missä se parhaiten kuvaa tämänhetkisen tilanteen:

paras mahdollinen _____ Pahin mahdollinen

Kun täytätte tämän kyselyn nyt, millaista todellista kiputilaa arvioisitte että vastauksenne vastaavat:

□ Vastaukset vastaavat tosi tilannetta, sillä koira ei ole syönyt kipulääkettä ollenkaan tai ei pitkään aikaan (yli 3 vrk)

□ Vastaukset osoittavat että koiran tila ehkä on hieman parempi kuin se olisi ilman mitään kipulääkettä, sillä se on ajoittain saanut kipulääkitystä

□ Vastaukset eivät välttämättä vastaa koiran todellista normaalia kiputilaa. Ne osoittavat todennäköisesti että koira on paremmassa kunnossa, sillä se on saanut kipulääkettä useasti

Koira sai (vaik. = vaikuttavaa)

□ edellisen kerran oikeata kipulääkitystä _____ tuntia /vrk /viikkoa / kk sitten

Mitä sai silloin?_____

<u>noin 4 viimeisen viikon kipulääkitykset:</u>

Koiralle viikoittain annettu kipulääkitys: Yksi viiva per antokerta.
(Käytä tukkimiehen kirjanpitoa: Yksi antokerta on yksi viiva. Jos koira ei ole saanut lääkettä, jätä tyhjäksi)

1 viikko sitten (ma-su): _____ antokertaa
2 viikkoa sitten (ma-su): _____ antokertaa
3 viikkoa sitten (ma-su): _____ antokertaa
4 viikkoa sitten (ma-su): _____ antokertaa

eli kuukauden aikana:

0 kertaa	1-2 kertaa	1 x /viikko	3-5 x / viikko	melkein päivittäin
□	□	□	□	□

<u>Muu hoito edellisen 30 päivän aikana:</u>

_____
_____
_____
_____
_____
_____

<u>Aikaisempi akupunktio hoito:</u>

Onko koira saanut akupunktiota aikaisemmin? kyllä □ ei □

Jos kyllä, mihin vaivaan?

_____

Auttoiko se? kyllä □ ei □

Mistä kuulitte Akupunktiosta? _____

Tällä kaavakkeella kartoitetaan koiranne yleistä hyvinvointia. Olkaa hyvä ja vastatkaa näihinkin kysymyksiin.

1. Ruokahalu on edellisen kuukauden aikana ollut:

erittäin hyvä	hyvä	tyydyttävä	huono	erittäin huono
□	□	□	□	□

2. Koira on edellisen kuukauden aikana oksennellut:

0 kertaa / kk	1-2 kertaa / kk	1 x / viikko	3-5 x / viikko	melkein päivittäin
□	□	□	□	□

3. Koira on edellisen kuukauden aikana ripuloinut:

0 kertaa / kk	1-2 kertaa / kk	1 x / viikko	3-5 x / viikko	melkein päivittäin
□	□	□	□	□

4. Koiralle on edellisen kuukauden aikana noussut iho-oireita ja/tai kutinaa

0 kertaa / kk	1-2 kertaa / kk	1 x / viikko	3-5 x / viikko	melkein päivittäin
□	□	□	□	□

5. Koira on edellisen kuukauden aikana saanut ravintolisiä (öljyjä, vitamiineja, nivelvalmisteita yms.)

0 kertaa / kk	1-2 kertaa / kk	1 x / viikko	3-5 x / viikko	melkein päivittäin
□	□	□	□	□

Mitä valmisteita ja miten usein _____
_____
_____
_____

Muuta huomioitavaa
_____
_____
_____

Kiitoksia vaivannäöstä! Tuokaa tämä sisään akupunktiolääkärille.

Lomakkeen voi myös postittaa meille osoitteeseen:
Anna Hielm-Björkman, ELT, CVA (IVAS)
Kliininen hevos- ja pieneläinlääketieteen laitos
Eläinlääketieteellinen tiedekunta
PL57, 00014 Helsingin Yliopisto, Suomi

tai lähettää sähköpostitse:
Sähköposti: anna.hielm-bjorkman@helsinki.fi

Appendix II

AKUPUNKTIO-KIPUPOTILAAN SEURANTA

Päiväys_____ Koiran nimi _____ Ikä _____ Sukup. ____

Koiran diagnoosi/ mistä hoidetaan? _____

Koiran lääkitys: _____

Edelliset hoidot ja pvm: _____

Koiran pääoireet? _____

Omistajan Nimi_____

Kaavakkeen täyttäjän allekirjoitus_____

Täyttöohjeet: Kirjoittakaa teksti viivoille. Voitte joko printata ja täyttää kynällä ja tuoda klinikalle tai postittaa, tai täyttää elektronisesti käyttämällä **X** ja **tummentamalla** oikea vastaus näin:

1. Mielentila on:

erittäin virkeä	**virkeä**	ei virkeä, eikä apea	apea	erittäin apea
□	X□	□	□	□

- Jokaiseen kysymykseen yksi vastaus. (Ei nolla eikä kaksi)
- Aina vastaatte joka kysymykseen samalla lailla joka kerta (esim. koira ehkä hyppää sohvaan ja autoon eri lailla jolloin on tärkeätä että aina vastaatte kysymykseen ajatellen samaa tilannetta ja että vastaatte aina esim pitkän lenkin jälkeen eikä ennen, jolloin vertaileva tilanne aina on sama).
- Sama ihminen / samat ihmiset vastaavat kyselyyn joka kerta, jolloin vastaukset ovat vertailukelpoisia
- Merkatkaa miten olette käyttäneet kipulääkkeitä
- Ilmoittakaa aina jos koiranne vahingossa on syönyt jotakin muuta lääkettä, ravintovalmisteita, hoitoa yms.

Kun täytätte tämän kyselyn nyt, millaista todellista kiputilaa arvioisitte että vastauksenne vastaavat:

□ Vastaukset vastaavat tosi tilannetta, sillä koira ei ole syönyt kipulääkettä ollenkaan tai ei pitkään aikaan (yli 3 vrk)

□ Vastaukset osoittavat että koiran tila ehkä on hieman parempi kuin se olisi ilman mitään kipulääkettä, sillä se on ajoittain saanut kipulääkitystä

□ Vastaukset eivät välttämättä vastaa koiran todellista normaalia kiputilaa. Ne osoittavat todennäköisesti että koira on paremmassa kunnossa, sillä se on saanut kipulääkettä useasti

Potilaan yleistila nyt

Rastita yksi vaihtoehto / kysymys; se joka parhaiten vastaa koirasi tilaa **menneellä viikolla**.

1. Mielentila on:

erittäin virkeä	virkeä	ei virkeä, eikä apea	apea	erittäin apea
□	□	□	□	□

2. Koira heiluttaa häntäänsä:

hyvin usein	usein	silloin tällöin	harvoin	hyvin harvoin
□	□	□	□	□

3. Koira leikkii:

hyvin mielellään	mielellään	vastahakoisesti	hyvin vastahakoisesti	ei ollenkaan
□	□	□	□	□

4a. Koira kävelee:

hyvin mielellään	mielellään	ei mielellään, eikä vastahakoisesti	vastahakoisesti	hyvin vastahakoisesti
□	□	□	□	□

4b. Koira kävelee:

erittäin helposti	helposti	kohtalaisesti	vaikeasti	hyvin vaikeasti
□	□	□	□	□

5a. Koira ravaa (siirtää ristikkäistä etu- ja takajalkaa samanaikaisesti):

hyvin mielellään	mielellään	vastahakoisesti	hyvin vastahakoisesti	ei ollenkaan
□	□	□	□	□

5b. Koira ravaa (siirtää ristikkäistä etu- ja takajalkaa samanaikaisesti):

erittäin helposti	helposti	vaikeasti	hyvin vaikeasti	ei ollenkaan
□	□	□	□	□

6. Koira peitsaa (siirtää samanpuoleista etu- ja takajalkaa samanaikaisesti):

hyvin harvoin	harvoin	silloin tällöin	usein	hyvin usein
□	□	□	□	□

7. Koira laukkaa:

hyvin mielellään	mielellään	vastahakoisesti	hyvin vastahakoisesti	ei ollenkaan
□	□	□	□	□

7b Koira laukkaa:

erittäin helposti	helposti	vaikeasti	hyvin vaikeasti	ei ollenkaan
□	□	□	□	□

8. Koiran tapa laukata muistuttaa takaa jänistä: molemmat takajalat liikkuvat yhdessä

hyvin harvoin	harvoin	silloin tällöin	usein	hyvin usein
□	□	□	□	□

9. Koira liikkuu oma-aloitteisesti ulkona:

hyvin mielellään	mielellään	vastahakoisesti	hyvin vastahakoisesti	ei ollenkaan
□	□	□	□	□

10. Koira liikkuu kovan rasituksen ja senjälkeisen levon jälkeen:

erittäin helposti	helposti	ei helposti, eikä vaikeasti	vaikeasti	hyvin vaikeasti
□	□	□	□	□

11a. Koira hyppää (esim. sohvaan, autoon tms.):

hyvin mielellään	mielellään	vastahakoisesti	hyvin vastahakoisesti	ei ollenkaan
□	□	□	□	□

11b. Koira hyppää (esim. sohvaan, autoon tms.):

erittäin helposti	helposti	vaikeasti	hyvin vaikeasti	ei ollenkaan
□	□	□	□	□

12. Koira kulkee rappusia ylös:

hyvin mielellään	mielellään	vastahakoisesti	hyvin vastahakoisesti	ei ollenkaan
□	□	□	□	□

13. Koira kulkee rappusia alas:

hyvin mielellään	mielellään	vastahakoisesti	hyvin vastahakoisesti	ei ollenkaan
□	□	□	□	□

14. Koira menee makuulle:

erittäin helposti	helposti	ei helposti, eikä vaikeasti	vaikeasti	hyvin vaikeasti
□	□	□	□	□

15. Koira nousee makuulta:

erittäin helposti	helposti	ei helposti, eikä vaikeasti	vaikeasti	hyvin vaikeasti
□	□	□	□	□

16. Koira läähättää kivun vuoksi:

ei juuri koskaan	harvoin	joskus	usein	hyvin usein
□	□	□	□	□

17. Koira valittaa kipuja:

ei juuri koskaan	harvoin	joskus	usein	hyvin usein
□	□	□	□	□

18. Koira valittaa kun takajalkoja venytetään taakse:

ei juuri koskaan	harvoin	joskus	usein	hyvin usein
□	□	□	□	□

19. Koira liikkuu kovan rasituksen jälkeen:

erittäin helposti	helposti	ei helposti, eikä vaikeasti	vaikeasti	hyvin vaikeasti
□	□	□	□	□

Koiran liikuntavaikeudet

Arvioi koiran liikkumavaikeus piirtämällä rasti alla olevalle janalle, siihen missä se parhaiten kuvaa tämänhetkisen tilanteen:

Ei mitään vaikeuksia _____ Pahin mahdollinen tilanne

Elämänlaatu

Arvioi koiran elämänlaatua piirtämällä rasti alla olevalle janalle, siihen missä se parhaiten kuvaa tämänhetkisen tilanteen:

paras mahdollinen _____ Pahin mahdollinen

Koiralle viimeisten viikkojen / kuukauden kipulääkitys:

Koira sai

□ edellisen kerran kipulääkitystä _____ tuntia /vrk /viikkoa / kk sitten

Mitä sai silloin?_____

Jos kävit **akupunktiossa 1-2 viikkoa sitten** edellisen kerran, niin miten olet antanut kipulääkkeitä:

0 kertaa/viikko	1-2 kertaa/viikko	3-4 kertaa/viikko	5-6 kertaa/viikko	päivittäin
□	□	□	□	□

Jos olet käynyt akupunktiossa edellisen kerran **yli kuukausi sitten**, (_____ viikkoa / kuukautta sitten) niin edellisen hoidon jälkeen olet antanut kipulääkkeitä keskimäärin:

0 kertaa	1-2 kertaa	1 x /viikko	3-5 x / viikko	melkein päivittäin
□	□	□	□	□

Mitä lääkettä sai silloin?_____

VERTAILEVA KYSELY

Seuravat kysymykset ovat vertailevia. Vertaatte koiranne nykytilaa siihen miten koira oli ennen kuin koiranne sai kultahippuhoidon / aloitti akupunktiohoidon:

Onko koiranne

	paljon parempi	vähän parempi	saman-lainen	vähän huonompi	paljon huonompi
Liikkuminen	☐	☐	☐	☐	☐
Rappusia ylös	☐	☐	☐	☐	☐
Rappusia alas	☐	☐	☐	☐	☐
Maaten alas	☐	☐	☐	☐	☐
Nousee ylös	☐	☐	☐	☐	☐
Ylöspäin kiipeily	☐	☐	☐	☐	☐
Hyppääminen	☐	☐	☐	☐	☐
Kävely	☐	☐	☐	☐	☐
Ravi	☐	☐	☐	☐	☐
Laukka	☐	☐	☐	☐	☐
Peitsaaminen	☐	☐	☐	☐	☐
Pupu-laukka	☐	☐	☐	☐	☐
Oma-aloitteinen liikkuminen	☐	☐	☐	☐	☐
Tilanteita:					
Levon jälkeen	☐	☐	☐	☐	☐
Kovan rasituksen jälkeen	☐	☐	☐	☐	☐
Rasitus + lepo, jälkeen	☐	☐	☐	☐	☐
Kipu - yleensä	☐	☐	☐	☐	☐
Kipu takajalkoja venyttäessä	☐	☐	☐	☐	☐
Läähättäminen	☐	☐	☐	☐	☐
Valittaminen kivusta	☐	☐	☐	☐	☐
Mielentila	☐	☐	☐	☐	☐
Sosiaalisuus	☐	☐	☐	☐	☐
Leikkisyys	☐	☐	☐	☐	☐
Elämänlaatu	☐	☐	☐	☐	☐
Turkki ja iho					
Turkin kiilto	☐	☐	☐	☐	☐
Turkin pehmeys	☐	☐	☐	☐	☐
Turkin tuuheus	☐	☐	☐	☐	☐
Ihon kunto	☐	☐	☐	☐	☐

Tällä kaavakkeella seurataan koirien yleistä hyvinvointia. Olkaa hyvä ja vastatkaa näihinkin kysymyksiin.

1. Ruokahalu on edellisen kuukauden aikana ollut:

erittäin hyvä	hyvä	tyydyttävä	huono	erittäin huono
□	□	□	□	□

2. Koira on edellisen kuukauden aikana oksennellut:

0 kertaa / kk	1-2 kertaa / kk	1 x / viikko	3-5 x / viikko	melkein päivittäin
□	□	□	□	□

3. Koira on edellisen kuukauden aikana ripuloinut:

0 kertaa / kk	1-2 kertaa / kk	1 x / viikko	3-5 x / viikko	melkein päivittäin
□	□	□	□	□

4. Koiralle on edellisen kuukauden aikana noussut iho-oireita ja/tai kutinaa

0 kertaa / kk	1-2 kertaa / kk	1 x / viikko	3-5 x / viikko	melkein päivittäin
□	□	□	□	□

5. Koira on edellisen kuukauden aikana saanut niveliin tai lihaksiin vaikuttavia ravintolisiä

0 kertaa / kk	1-2 kertaa / kk	1 x / viikko	3-5 x / viikko	melkein päivittäin
□	□	□	□	□

Mitä ja miten usein _____
_____
_____

Muuta (esim. muita hoitoja, lääkkeitä, uusia oireita, mikä parantunut?...)
_____
_____
_____
_____
_____

Kiitoksia vaivannäöstä!