EFFECTS OF ELECTROACUPUNCTURE IN A MOUSE MODEL OF EXPERIMENTALLY-INDUCED OSTEOSARCOMA

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DEDICATION

To my husband, Scott Jenquin,
for his love and reassurance,
and for keeping me grounded.

ABSTRACT

Osteosarcoma (OSA) is a devastating form of musculoskeletal cancer that most commonly results in death due to pulmonary metastatic disease. It is a rapidly growing, aggressive bone neoplasm, which accounts for up to 85% of all malignant bone tumors in small animals and about 400 new cases per year in humans. Clinically, acupuncture and electroacupuncture (EA) have been used in human and veterinary medicine mainly as adjunct therapies. The anti-inflammatory, immune-boosting, and analgesic effects of electroacupuncture (EA) are well-documented in a variety of animal models and human patients. A number of studies suggest that females have lower pain thresholds than males. In this regard, central c-fos expression was higher in females with chronic pain syndromes, and recent experiments in our lab suggest a trend toward differential spinal cord c-fos expression in tumor-bearing females versus males. However, males are more predisposed to developing OSA, and because of this susceptibility, it is to be expected that they exhibit faster growing, larger, and more painful tumors than females. To date, there are no studies investigating the gender effects of EA on OSA pain and tumor growth. Moreover, EA has never been investigated as sole therapy in a mouse model of bone cancer.

We studied the effects of EA in Balb-C mice, by implanting K7M2 osteosarcoma cells into the calcaneus bone of the left hind paw. Electroacupuncture (4 Hz) was applied to the Zusanli (ST-36) acupuncture point at different time intervals. EA+1: once, 24 hours after tumor implantation. EA+: once weekly for 3 weeks. EA++: twice weekly for 3 weeks, starting on day 3 post-implantation. EA+5: twice weekly for 3 weeks, starting on day 5 post-implantation. EA+7: twice weekly, starting on day 7 post-implantation. PxEA+: 3 treatments prior to implantation. Each group was accompanied by a sham treatment group (no current). Primary hyperalgesia was evaluated using von Frey filaments. Spinal samples underwent avitin-biotin/DAB immunohistochemistry to quantify c-fos expression using light microscopy. Tumor size was measured

using calipers. Tumor tissue innervation and vascularization were also analyzed using confocal microscopy. Statistical comparisons between groups were carried out using a Repeated Measures ANOVA ($p \leq 0.05$) followed by post-hoc Bonferroni analysis where necessary. Primary hyperalgesia was evaluated using von Frey filaments applied to the plantar surface of each paw. Tumor size was measured using calipers. Vaginal swabs were carried out in female mice to determine the stage of the estrous cycle during treatments.

Tumors in control animals showed a 30.23% growth increase from baseline, while EA+ animals exhibited an average tumor growth increase of 100% or more. Significant reductions in tumor size were seen in mice undergoing early and frequent EA treatments (EA++). Hyperalgesia consistently increased with tumor growth, although less so in EA-treated mice. Hyperalgesia dropped slightly in both males and females on the days EA was performed, but rose again 24 hours later. Estrous cycles in female mice varied greatly and were not synchronized within groups. They showed no correlation to EA or behavioral testing, indicating no direct hormonal influence. Tumors tended to grow slightly larger in males and resulted in higher von Frey scores and c-fos expression across the groups. With few exceptions, there were no significant gender differences in tumor growth or von Frey scores.

The results of this study indicate that early EA treatment has inhibitory effects on nociception and tumor growth that are not influenced by gender, while late EA treatment actually increases tumor growth.

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I. PURPOSE OF STUDY

The purpose of this study was to examine the effects of electroacupuncture administered at the ST-36 (Zusanli) acupuncture point on tumor growth and tumor-induced nociception. It served to assess the analgesic effectiveness of electroacupuncture for treating bone cancer pain. In addition, it provided a means to evaluate electroacupuncture as a novel and singular treatment alternative to conventional cancer therapy. The new knowledge gained in this study was achieved by correlating the effects of electroacupuncture with gross changes in tumor size and localized innervation and vascularization at the tumor site, as well as correlating mechanical hypersensitivity responses with the biomarker c-fos, an oncogene protein that represents the best global marker for efficiently locating populations of neurons in the awake animal that respond to nociceptive input (Coggeshall, 2005).

II. SPECIFIC AIMS AND HYPOTHESES

The research presented in this thesis was focused on addressing the following questions:

1. **Does electroacupuncture affect tumor growth in mice with experimentally-induced osteosarcoma?**

   More specifically, this research was designed to elucidate if electroacupuncture had a tumor-enhancing or tumor-inhibiting effect, and if so, whether this was due to changes in tumor innervation or vascularization.

2. Does electroacupuncture reduce nociception in mice with experimentally-induced osteosarcoma?

The research was focused on discovering if different electroacupuncture regimens had differential effects on tumor-induced nociception.

3. Does gender influence electroacupuncture effects on tumor growth and nociception in mice with experimentally-induced osteosarcoma?

The gender issue arose because males have been reported to have a slight predilection for developing osteosarcoma, but females reportedly have lower pain thresholds and increased tumor-induced hypersensitivity. Moreover, females have been reported to use acupuncture treatment more often than males (Ben-Arye et al., 2009). Thus, the experimental design was developed to address the question of gender effects on acupuncture.

The work presented in this thesis is original and there is little literature available that addresses acupuncture and cancer. Consequently, it was impossible to predict exactly what results to expect. However, based on certain documented shared biological mechanisms, it was possible to link electroacupuncture and osteosarcoma, and thus, design the experiments in this thesis with the following aims and hypotheses in mind.

1. Does electroacupuncture affect tumor growth in mice with experimentally-induced osteosarcoma?

Aim 1: To determine whether electroacupuncture has a tumor-enhancing or tumor-inhibiting effect and whether this effect is dependent on specific electroacupuncture treatment strategies.

Hypothesis 1: The inducible cyclooxygenase-2 (COX-2) is overexpressed in canine (Mullins et al., 2004) and human (Masi et

al., 2007) osteosarcoma cells, implying an inflammatory link between inflammation and tumerogenesis (Naruse et al., 2006; Lin and Karin, 2007). Electroacupuncture, a proven COX-2 inhibitor (Lee et al., 2005a; Lee et al., 2006; Lau et al., 2008) with anti-inflammatory (Kim et al., 2006) and immune-boosting properties (Yu et al., 1998b), has the potential to interfere with osteosarcoma growth and development. In this regad we hypothesize that electroacupuncture treatment at ST-36 will reduce tumor size based on its anti-inflammatory effect.

Hypothesis 2: Electroacupuncture is considered to be a more potent treatment modality than sham or traditional acupuncture (Chiu et al., 2003). Thus, we hypothesize that electroacupuncture treatment will have a greater anti-tumor effect than non-electrical stimulation.

Aim 2: To determine whether electroacupuncture causes changes in the innervation and vascularization patterns of osteosarcoma tumors in the calcaneous bone of the hindpaw.

Hypothesis: Tumor metastasis and progression are highly dependent on vasculature (Bacci et al., 1998; Harris et al., 1998; Hansen-Algenstaedt et al., 2005; Fuhrhop et al., 2009). Tumor growth is also associated with changes in innervation (Wacnik et al., 2005a). Electroacupuncture induces a variety of peripheral vascular effects (Nappi et al., 1982; Ohsawa et al., 1995; Wacnik et al., 2005a) and changes in nerve innervation (Takeshige and Sato, 1996; Carlsson et al., 2006). We hypothesize that electroacupuncture will reduce tumor vascularization and innervation and that this is one of the mechanisms that result in reduce tumor growth.

Aim 3: To determine whether electroacupuncture affects tumor metastasis to the lungs.

Hypothesis: Even though lung metastases are the inevitable consequence of an established tumor (Peltier, 1993; Link and Eilber, 1997), to date there have been no studies that elucidate the effect of either acupuncture or electroacupuncture on tumor metastasis. However, it stands to reason that if electroacupuncture has the potential to interfere with tumor growth – as described above, then it is also likely to interfere with the tumor's ability to seed metastases. Recent work also indicates that tumor metastasis is influenced by inflammation at the tumor site. Based on this, we hypothesize that electroacupuncture will reduce lung metastases in part by reducing inflammation at the tumor site.

2. Does electroacupuncture reduce nociception in mice with experimentally induced osteosarcoma?

Aim 4: To determine whether electroacupuncture applied at ST-36 is effective in reducing osteosarcoma-induced mechanical hyperalgesia. This aim stems from the reported analgesic and anti-inflammatory properties of acupuncture and electroacupuncture.

Hypothesis 1: Tumor growth is associated with changes in innervation, specifically the increased number of calcitonin-gene related peptide (CGRP)-expressing nerve fibers (Wacnik et al., 2005a). Furthermore, the ability of osteosarcomas to express COX-2 (Mullins et al., 2004; Masi et al., 2007), as well as other nociceptive mediators such as TNFα (Wacnik et al., 2005b), endothelin (Wacnik et al., 2001), nerve growth factor (McMahon, 1996; Sevcik et al., 2005), indicate that inflammatory pain is a significant component of tumor-induced pain. Electroacupuncture

produces multimodal analgesic effects, such as the activation of descending anti-nociceptive pathways and inhibition of multiple limbic areas (Wu et al., 1999), the release of endogenous opioids both centrally and in the periphery (Zhang et al., 1980; Xi et al., 1983), as well as the inhibition of proinflammatory COX-2 (Kim et al., 2006), TNFα and IL-6 (Kavoussi and Ross, 2007). It also produces anti-nociceptive effects in line with the gate control theory of pain (Ernst and Lee, 1987). We hypothesize that electroacupuncture presents a novel multimodal analgesic modality that will decrease tumor-induced nociception via an action on nerves at the acupoint site that convey acupuncture information to the spinal cord to activate local inhibitory circuits as well as descending noxious inhibitory control pathways.

Hypothesis 2: Spinal and cranial c-fos expression was successfully correlated to pain transmission and to acupuncture activation (Lee and Beitz, 1992 and 1993; Harris, 1998). Based on the multimodal analgesic mechanism mentioned above and the reported direct inhibitory effect on cranial c-fos expression (Lee and Beitz, 1992), we hypothesize that electroacupuncture will decrease tumor-induced c-fos expression in the spinal cord dorsal horn.

3. Does gender influence electroacupuncture effects on tumor growth and nociception in mice with experimentally induced osteosarcoma?

Aim 5: To determine whether electroacupuncture analgesia is more pronounced in male versus female mice with osteosarcoma-induced nociception, and whether these gender differences in electroacupuncture analgesia are due to the influence of sex hormones.

Hypothesis 1: The studies on possible gender-related analgesic effects of acupuncture, electroacupuncture, or transcutaneous electrical nerve stimulation (TENS) are sparse and contradictory (Lund and Lundeberg, 2008). Even though males exhibit a slight predisposition to develop osteosarcoma (Turrel and Pool, 1982; Carpenter et al., 1987), females are attributed with having lower pain thresholds than males (Berkley, 1997; Fillingim and Ness, 2000; Craft et al., 2004). We hypothesize that acupuncture will be more effective in male mice than female mice, because female mice will exhibit higher tumor-induced nociceptive scores and higher blood estrogen levels.

Hypothesis 2: Since males are predisposed to develop osteosarcoma (Turrel and Pool, 1982; Carpenter et al., 1987), we hypothesize that male mice will exhibit larger sized tumors than females regardless of treatment.

III. SIGNIFICANCE OF STUDY

This thesis represents the first study to explore the effects of electroacupuncture as a novel treatment alternative for osteosarcoma and osteosarcoma-associated pain.

Acupuncture and electroacupuncture are gaining increased popularity as supplemental or supportive therapies, complementing conventional cancer treatments (Cassileth et al., 2007; Minton and Higginson, 2007; Lu et al., 2008; Mao-Ying et al., 2008). However, neither manual acupuncture nor electroacupuncture administered at ST-36 has been evaluated for its potential anti-tumor effects in a mouse model of bone cancer. A recent PubMed search yielded only one abstract of a study published in Chinese that investigated the

effects of electroacupuncture on tumor growth and immune function in rat models of liver and gastric cancer, as well as hypodermic tumors (Lai et al., 2008). In this study, low-frequency electroacupuncture was administered at ST-36 and other acupuncture points for 15 minutes once daily over a period of 15 days. The results indicate that the treatment was effective in increasing immune function (as indicated by increased levels of IgG and IgM immunoglobulins, as well as C4+ and CD4+/CD8+ immune cells) and inhibiting tumor growth. However, in the absence of an English language translation and consequent access to details about methodology (specifically onset of electroacupuncture sessions after tumor implantation) and results, it is difficult to scientifically evaluate either the outcome or merits of this study. A PubMed search into the use of (non-complementary) electroacupuncture in cancer conditions yielded 2 further studies by a group of investigators at the University of Maryland investigating the analgesic effects of electroacupuncture administered at the GB30 acupuncture point in a rat model of bone cancer pain. In both cases, daily treatments commenced between days 14 and 18 post-tumor implantation, and the study focused on the effects of acupuncture in attenuating bone-cancer-induced hyperalgesia via the inhibition of spinal preprodynorphin (Zhang et al., 2008) and spinal interleukin-1β (Zhang et al., 2007c).

Based on a careful search of the existing literature, it is clear that there is a deplorable lack of well-controlled and standardized studies evaluating electroacupuncture as a stand-alone alternative analgesic or anti-tumor therapy. This thesis represents the first study investigating electroacupuncture as the sole treatment modality for the primary bone cancer osteosarcoma and the associated pain that it induces in a mouse bone cancer model.

IV. INTRODUCTION

Osteosarcoma is a devastating form of musculoskeletal cancer that most commonly results in death due to pulmonary metastatic disease (Peltier, 1993; Link and Eilber, 1997). The biological and genetic mechanisms that contribute to the development of this type of cancer are still not completely understood. Consequently, current medical therapies are radical and prognoses remain guarded. Looking beyond the limits of Western medicine and towards alternative and Eastern medical treatments provides an entirely new perspective and novel opportunity to study and potentially treat this destructive cancer. The purpose of this thesis was to examine the effect of acupuncture treatment on osteosarcoma growth, metastasis, innervation and vascularization.

A. Osteosarcoma

Osteosarcoma is the most common type of bone sarcoma, which mainly affects adolescents and young adults (Akiyama et al., 2008; Weber et al., 2008). The incidence of osteosarcoma is approximately 400 cases per year in the human population of the United States (Ries et al., 1999). The overall 5-year survival rate for patients diagnosed between 1974 and 1994 was 63% (59% for males, 70% for females) (Ries et al., 1999). In dogs and cats, skeletal neoplasms have an incidence of 7.9/100,000 (Misdorp and Hart, 1979). Ninety-eight percent of these are considered malignant and may be classified as being either primary or metastatic. The majority of primary bone tumors are osteosarcomas (Straw, 1996). Skeletal neoplasms are more common in dogs than in any other species, with primary and malignant tumors outnumbering all other types (Slayter et al., 1994). Osteosarcoma is the most common primary bone tumor in dogs. It is a rapidly growing, aggressive bone neoplasm that accounts for approximately 85% of all malignant bone tumors (Theilen and Madewell, 1987; Pool, 1990; Ogilvie, 2001). Overall, osteosarcoma represents approximately 3-6% of all canine

neoplasms, with roughly 10,000 new cases of osteosarcoma diagnosed each year (Chun and de Lorimier, 2003). The incidence of skeletal neoplasms in cats is 4.9/100,000, of which 67-90% may be malignant (Belito et al., 1987). Osteosarcomas represent 70-80% of all primary bone tumors and 5-7% of all feline neoplasms. It is important to note that this neoplasm targets older animals and appears to have no breed predilection (Belito et al., 1987). Males also seem to be more predisposed to develop osteosarcomas than females, but these reports are controversial (Turrel and Pool, 1982; Carpenter et al., 1987). Osteosarcomas are highly metastatic and the most common metastatic sites are the lungs and lymph nodes. Metastasis occurs by primary invasion of veins and subsequent embolization to the lungs (Peltier, 1993; Link and Eilber, 1997).

Since the original description of this disease (Peltier, 1993), researchers have carried out a great deal of both basic science and clinical research to study and understand osteosarcoma. However, while we now have an increased capability to diagnose and evaluate osteosarcoma, we still do not have a full understanding of the biological and genetic mechanisms that contribute to the development of this type of cancer. Recent work has shown that upregulation of the cell cycle and downregulation of Wnt signaling have an important role in osteosarcoma genesis (Cleton-Jansen et al., 2009. However, while a number of recent genomic and proteomic studies have identified altered genes and protein expression in osteosarcomas (Cleton-Jansen et al., 2009; Daino et al., 2009; Folio et al., 2009; Selvarajah et al., 2009; Thomas et al., 2009), we are still far from a solid understanding of the mechanisms that lead to the development of osteosarcomas and to the development of osteosarcoma-induced pain.

Surgical excision still remains the mainstay of therapy in both humans and small animals (Shenoy et al., 2008). Moreover, while the combination of modern surgery and systemic chemotherapy has improved the treatment of osteosarcoma (Akiyama et al., 2008), chemotherapy alone does not significantly affect survival rate (Shenoy et al., 2008). Despite the strides made in osteosarcoma treatment and diagnosis, our lack of understanding of the basic

mechanisms leading to the development of osteosarcoma has prevented any substantial change in treatment or survival over the past 20 years (Akiyama et al., 2008). Thus, the long-term prognosis for both canine and human patients with osteosarcoma is guarded, and their life expectancies are relatively short. In this regard, the prognosis for patients is highly dependent on a number of factors including the size and location of the osteosarcoma, whether it is primary, metastatic or recurrent, the tumor stage, the patient's age and general health, and particularly in the case of small animals, the histological grade, surgery and mitotic index (Dimopoulou et al., 2008).

B. Acupuncture and Electroacupuncture

Acupuncture is a popular complementary and alternative medical modality that is used to treat a wide variety of medical conditions both in humans and animals (Chan et al., 2001; Lee et al., 2005b). Historically, the earliest written reports on the use of acupuncture by Chinese physicians date back to the period of 480-220 BC. However, there is evidence to suggest that it was practiced as early as 4,500 years ago (Wu, 1996). Acupuncturists stimulate specific locations on the body (acupoints) using needles (acupuncture), touch (acupressure), or heat (moxibustion). Traditionally, acupuncture was practiced in the context of interactive diagnostic procedures based on complex philosophical laws. One of the key Eastern Medicine philosophical concepts underlying the effectiveness of acupuncture centers on stimulating the Qi (life force or energy), which is thought to flow through the body. In healthy individuals the Qi is balanced between the two major forces, Yin and Yang. The Qi flows along meridians, which are precisely mapped channels that connect to each other and to the various body organs (Mayer, 2000). When the energy flow along these meridians is out of balance, disease develops. Acupuncture stimulation of specific points along the meridians is thought to restore the proper energy balance. Once considered purely a philosophical concept, scientific evidence suggests that meridians are

real and consist of blood vessels (traditional), sheets of connective tissue (Langevin et al., 2002; Langevin and Yandow, 2002), or nerves (Li et al., 2004).

While early reports on the positive effects of acupuncture on pain and certain disease processes were primarily anecdotal, recent controlled studies suggest that acupuncture can indeed relieve pain, reduce inflammation and successfully treat certain medical conditions (Napadow et al., 2008). For instance, recent systematic reviews of the effect of acupuncture on chronic headaches concluded that acupuncture could be a valuable non-pharmacological treatment tool (Linde et al., 2009), and that needling acupuncture is superior to sham acupuncture and medication therapy in improving headache intensity, frequency, and response rate (Sun and Gan, 2008). There is also strong evidence that acupuncture can be a useful supplement to other forms of conventional therapy for nonspecific lower back pain (Yuan et al., 2008) and for the treatment of postoperative pain (Sun et al., 2008). Further support for the effectiveness of acupuncture for back pain in dogs with thoracolumbar intervertebral disk disease showed that electroacupuncture combined with standard Western medical treatment was effective and resulted in shorter time to recover than did use of Western treatment alone (Hayashi et al., 2007). Similarly, recent research suggests that acupuncture is an important therapy for treating neuropathic pain in dogs and cats (Mathews, 2008) and humans (Taguchi, 2008). On the other hand, many systematic reviews of clinical acupuncture studies fail to find convincing evidence that acupuncture relieves pain (Ee et al., 2008; Madsen et al., 2009; Tough et al., 2008; Wang et al., 2008a). For instance, Pittler and Ernst (2008) recently concluded that, “the evidence is not fully convincing for most complementary and alternative medicine modalities in relieving neuropathic or neuralgic pain”. The problem with most clinical studies of acupuncture to date relate to methodological issues, including limited sample sizes, lack of randomization, inappropriate control groups, and discounting the concept of Eastern Medicine philosophy.

The use of electrically stimulated needles was first reported in the 1960s (Sheng and Chang, 1960; Schwarz, 1966). This electroacupuncture provided the statistically most significant surgical analgesia (Leong and Chernow, 1988) when compared to traditional acupuncture manual stimulation procedures. The last three decades have seen a surge of renewed scientific interest in acupuncture and electroacupuncture. Numerous studies strived to identify central and peripheral effects of electroacupuncture and acupuncture (Nappi et al., 1982; Ohsawa et al., 1995; Yu et al., 1998b; Wacnik et al., 2005a). Several have focused on the possibility that acupuncture stimulates endogenous opioids (Zhang et al., 1980; Xi et al., 1983; Han et al., 1984). In this regard, at least three separate endogenous opioid neuronal systems exist in the brain: enkephalins, β-endorphins and dynorphins. Acupuncture increases endogenous opioids both centrally and peripherally. For example, in patients suffering from brain tumors, acupuncture increases the level of opioids in the cerebrospinal fluid (Zhang et al., 1980). Increases in serum opioids also occur following acupuncture in patients with soft tissue pain, acute appendicitis and peri-arthritis (Xi et al., 1983). Injection of endorphin antibodies against Met-enkephalin or β-endorphin into the periaqueductal gray (PAG) in rabbits resulted in decreases in the analgesic effect of electroacupuncture (Han et al., 1984). Moreover, naloxone, a specific opioid antagonist, repeatedly reverses the analgesic effects of acupuncture (Pomeranz and Chiu, 1976; Mayer et al., 1977). Electroacupuncture also produces an immediate, segmental analgesia of short duration that is not mediated by endogenous opioids, but rather has been postulated to be based on the gate control theory (Ernst and Lee, 1987). It is of interest that one recent theory indicates that acupuncture works through potentiation and modulation of a highly organized and somatotopic network of endogenous opioids that links expectation, attention and body schema (Liu, 2008). In a recent review of the neural mechanisms underlying acupuncture analgesia, Zhao (2008) states that, “acupuncture analgesia is a manifestation of integrative processes at different levels of the central nervous system (CNS) between afferent impulses from pain regions and impulses from acupoints”. He goes on to say that, “opioid peptides in

the arcuate-PAG-NRM-spinal dorsal horn pathway play a pivotal role in mediating acupuncture analgesia". Thus, acupuncture and electroacupuncture induce their anti-nociceptive effects by several mechanisms resulting in a multimodal analgesia. This implies that either treatment represents strong competition to Western pharmacological pain intervention.

C. Linking Electroacupuncture and Cancer

One of the most important complications of osteosarcoma is cancer-induced bone pain, which is present in both canine and human patients.

Except possibly for bisphosphonates, which provide pain relief by delaying the progression of bone lesions, there are no current pharmacotherapeutical approaches to bone cancer pain that effectively relieve pain without the development of side effects or tolerance to the drug, particularly in terminal cancer patients (Fan et al., 2007). There is no better reason to look beyond existing treatment modalities than their limitations. We need to explore non-traditional and novel ideas, if we want to find new options in cancer and pain therapy. A recent evidenced based review concluded that, "a great body of data emerging from scientifically sound clinical trials proves that defined complementary procedures are beneficial for oncology patients" (Beuth and Schierholz, 2007). However, it is important to differentiate between "alternative" therapies, often promoted falsely as viable options to mainstream cancer treatment, and complementary therapies, adjunctive, effective techniques that treat symptoms associated with cancer and its mainstream treatment. In this regard, both Cassileth et al. (2007) and Lu et al. (2008) concluded in recent reviews that complementary therapies, including acupuncture, play an increasingly important role in the control of symptoms, such as pain, associated with cancer and cancer treatment. Thus, from a clinical standpoint, needling acupuncture and electroacupuncture are advocated in both human and veterinary medicine as adjunct therapies to treat the adverse effects of cancer treatment, or to contribute to multimodal pain management (Staud and Price,

2006). In support of this approach Zhang et al. (2008) showed that electroacupuncture significantly reduced both thermal hyperalgesia and mechanical hyperalgesia in a rat model of pain induced by prostatic bone cancer. Conversely, neither electroacupuncture treatment alone, nor treatment with Celebrex (at a dose of 5 mg/kg/d) alone, had any effect on bone tumor-induced mechanical allodynia in a rat model of tibial mammary carcinoma (Mao-Ying et al., 2008). However, when electroacupuncture was combined with 5 mg/kg Celebrex their combined use significantly reduced tumor-induced allodynia. These studies suggest that further research is necessary to verify that acupuncture is effective in treating bone cancer pain and to discover the mechanisms underlying the effectiveness of electroacupuncture. Thus, it was an important aspect of this thesis to investigate whether electroacupuncture could effectively reduce mechanical hyperalgesia in a murine hindpaw model of osteosarcoma-induced pain.

Electroacupuncture applied to the Zusanli (ST-36) acupuncture point produces analgesic effects in a rat model of mechanical hyperalgesia (Lee and Beitz, 1992) and in a mouse model of chemical hyperalgesia (Chang et al. 2004). The same acupoint is popular for its anti-inflammatory effects as demonstrated by Kim et al. (2006) in their murine model of air pouch inflammation. In addition, electroacupuncture at ST-36 affects interferon-γ levels and subsequently natural killer (NK) cell activity, which suggests immune-boosting effects (Yu et al., 1998b). Recently, inflammation was linked to tumerogenesis when selective COX-2-inhibiting nonsteroidal anti-inflammatory drugs, such as meloxicam, exhibited antineoplastic effects in vitro (Naruse et al., 2006; Wolfesberger et al., 2006). Lin and Karin (2007) extensively reviewed this extraordinary finding. In effect, the inducible enzyme COX-2 is overexpressed in canine (Mullins et al., 2004) and human (Masi et al., 2007) osteosarcoma cells, and electroacupuncture significantly decreases COX-2 expression in different models of pain, hypersensitivity (Lau et al., 2008) and inflammation (Lee et al., 2005a; Lee et al., 2006). In addition, in the periphery, deep acupuncture stimulation at ST-36

induces a decrease in sympathetic renal nerve activity and mean arterial blood pressure in anesthetized rats (Ohsawa et al., 1995).

Electroacupuncture applied at the ST-36 acupuncture point also significantly raises the plasma levels of β-endorphin, β-lipotropin and adrenocorticotropic hormone (ACTH) (Nappi et al., 1982). Therefore, it is conceivable that any changes in circulation may affect tumor growth. Moreover, in hyperalgesic mice, tumors tend to exhibit increased CGRP-immunoreactive nerve fibers and reduced vascularization (Wacnik et al., 2005a). Using a pain model of tetanized gastrocnemius muscle in guinea pigs, Takeshige and Sato (1996) found that needling of the muscle stimulated a variety of sensory nerve endings, including those containing CGRP. Twice-weekly treatments with acupuncture reduced the number of CGRP-immunoreactive nerve fibers in the skin being needled, indicating that the pain-relieving effects of acupuncture depend in part on peripheral innervation (Carlsson et al., 2006).

Thus, it is quite possible that electroacupuncture has the ability to affect local vascularization and innervation associated with tumor growth. However, neither acupuncture nor electroacupuncture have been investigated adequately as sole therapies for cancer pain. More importantly, there are no studies in the literature that evaluate the effect of electroacupuncture on osteosarcoma growth and metastasis. Therefore, one of the goals of the experiments described in this thesis was to evaluate acupuncture as a potential treatment option for osteosarcoma-induced pain.

2. OSTEOSARCOMA

Although the disease was known since antiquity, the term "sarcoma" was not used in medical terminology until 1804, when it was introduced by the English surgeon John Abernathy, who derived the word from Greek roots meaning "fleshy excrescence" (Peltier, 1993). In 1805, the term “osteosarcoma” was coined by Napoleon’s personal surgeon, Alexis Boyer, who realized that osteosarcoma was distinctly different from other bone lesions (Peltier, 1993; Rutkow, 1993). He considered the osteosarcoma to be “a true cancerous degeneration of bone” (Peltier, 1993). This finding opened the doors to more organized and purposeful investigation of the disease. In 1847, the Baron Guillaume Dupuytren demonstrated his intimate knowledge of the gross pathologic appearance of osteosarcoma when he wrote the following:

> "Osteosarcoma, which is a true cancerous degeneration of bone, manifests itself in the form of a white or reddish mass, lardaceous and firm at an early stage of the disease; but presenting at a later period, points of softening, cerebriform matter, extravasating blood, and white or straw colored fluid of a viscid consistence in its interior." (Peltier, 1993)

The 20th century witnessed great endeavors to build organizations and programs dedicated to studying cancer. In 1913, the American Cancer Society was founded as the American Society for the Control of Cancer by a group of well-known doctors and business leaders in New York City[1]. This was followed by the creation of the Registry of Bone Sarcomas by Ernest Amory Codman in 1921, which represented a significant step forward into the modern age of medicine (Codman, 1924 and 1926). It provided valuable information to individual surgeons and physicians, who until then had limited resources to guide them in diagnosing, understanding, and treating these daunting tumors. Since then, great strides have been taken to study and understand osteosarcomas. Nearly 20

[1] www.cancer.org

years later the creation of the National Cancer Institute (NCI) was authorized by the U.S. Congress in the National Cancer Act of 1937[2]. By the mid 1900s, Henry L. Jaffe and his colleague Louis Lichtenstein published seminal textbooks devoted to bone pathology, establishing nearly all of the key histological criteria used to diagnose bone tumors (Jaffe, 1958; Lichtenstein, 1959). In the early 1970s, the NCI created a program dedicated to providing information on cancer statistics called the "Surveillance, Epidemiology and End Results (SEER) Program"[3]. The data generated by this program provides key information on the epidemiology of cancers in the United States.

However, despite all these efforts to gain better insights into the disease of cancer, osteosarcoma remains an incompletely understood neoplasm.

I. INCIDENCE

From 1975-1995, the SEER Program collected data to establish a compendium of statistical trends of childhood cancers and associated risks[4]. Osteosarcomas represented about 56% of primary bone tumors diagnosed in 1,657 children over the 21-year period (Ries et al., 1999). In the US, approximately 1,000 to 1,500 new cases of osteosarcoma are diagnosed annually, 400 of which are in children (Ries, et al. 1999). More recent numbers are higher, reporting as many as 560 new cases of osteosarcoma diagnosed annually in children and adolescents (Messerschmitt et al., 2009). In dogs the numbers are dramatically higher still: 10,000 new canine cases are diagnosed each year (Chun and de Lorimier, 2003).

[2] www.cancer.gov
[3] http://seer.cancer.gov/index.html
[4] http://seer.cancer.gov/publications/childhood/

Skeletal neoplasms in small animals have an incidence of 7.9/100,000 (Misdorp and Hart, 1979). Ninety-eight percent of these are considered malignant, and may be classified as being either primary or metastatic. The majority of primary bone tumors are osteosarcomas (85%) and to a lesser extend (5 to 10%) chondrosarcomas, fibrosarcomas, and hemangiosarcomas (Straw, 1996). Skeletal neoplasms are more common in dogs than in any other species, with primary and malignant tumors outnumbering all other types (Slayter et al., 1994). Osteosarcoma is the most common primary bone tumor in dogs. It is a rapidly growing, aggressive bone neoplasm that accounts for approximately 85% of all malignant bone tumors (Theilen and Madewell, 1987; Pool, 1990; Ogilvie, 2001). Overall, osteosarcoma represents approximately 3-6% of all canine neoplasms (Chun and de Lorimier, 2003). Osteosarcomas represent 70-80% of all primary bone tumors and 5-7% of all feline neoplasms. The incidence of skeletal neoplasms in cats is 4.9/100,000, 67-90% of which may be malignant (Belito et al., 1987).

II. Age and Sex Distribution

In humans, osteosarcoma occurs predominantly in children and adolescents, although a second peak of incidence is seen in adults older than 65 years of age (Miller et al., 1996). Seventy-five percent of all juvenile bone cancer cases, including osteosarcoma, arise between the ages of 10 to 19 years (Ries et al., 1999). The data compiled by SEER indicates that from 5 to 10 years of age, a steady rise of incidence rate was observed, which became a steeper rise beginning at age 11, with peak incidence at 15 years of age (Ries et al., 1999). It appeared that the rising incidence rates as children grew older coincided with their pubescent growth spurt. Similarly, this may also explain why large breed

dogs are more susceptible than those of smaller breeds (Theilen and Madewell, 1987; Pool, 1990).

In small animals, the age distribution is quite different. The mean age of occurrence of canine osteosarcomas is 7.5 years, but younger animals are frequently affected also (Theilen and Madewell, 1987; Pool, 1990). In cats, the mean age of osteosarcoma occurrence is 10 years, although it may range from 1-20 years (Turrel and Pool, 1982).

Males exhibit a slightly higher incidence rate of osteosarcoma than females, with the male to female incidence ratio ranging from 1.1:1 (Al-Nasrallah et al., 2007) to 1.39:1 (Dahlin and Unni, 1986) to 1.5:1 (Rosen et al., 2000). Even though the incidence pattern is similar for both genders, SEER reported some differences. Specifically, females reached the peak of cancer incidence at age 13, while males reached their highest rates after age 15. Male dogs also appear to be slightly more predisposed to develop osteosarcoma than females with a male to female ratio of 1.2:1 (Theilen and Madewell, 1987; Pool, 1990). In cats, the numbers are comparable (1.19:1) (Heldman et al., 2000). However, just as in humans, the reports about male predilection are inconsistent and the ratios vary (Turrel and Pool, 1982; Carpenter et al., 1987).

III. ETIOLOGY

Unfortunately, the exact etiology of osteosarcoma is still unknown (Kunze et al., 2009). In the SEER program report, Ries et al. (1999) state that “the current state of knowledge regarding the causes of bone cancer is limited. Although directed ionizing radiation exposure and a few genetic susceptibility syndromes are associated with increased risk of osteosarcoma, to date no factor has emerged to explain even a modest proportion of cases.”

A. Known Risk Factors

Cancer and radiation related. The risk for developing osteosarcoma was increased in cancer survivors following radiotherapy for childhood cancer or treatment with alkylating agents (Tucker et al., 1987; Newton et al., 1991; Hawkins et al., 1996). Adults exposed to high doses of the radioisotope radium are also at increased risk (Finkelstein and Kreiger, 1996; Miller et al., 1996).

Radiation-induced osteosarcoma is a common occurrence. For example, in a study investigating 87 dogs undergoing radiation treatment for soft-tissue sarcomas, 3.4% developed osteosarcoma 1.7 to 5 years post treatment (Gillette, 1990). An earlier study found 1 in 4 dogs receiving radiation therapy for oral epulides developed osteosarcoma after 78 months (Thrall, 1981).

Genetic. Osteosarcoma is a spontaneously occurring neoplasm, although there is evidence of hereditary or genetically determined susceptibility. The increased frequency of osteosarcomas in association with certain genetic disorders, such as hereditary retinoblastoma (Hansen et al., 1985; Wong et al., 1997) and Li-Fraumeni syndrome (Li et al., 1988), has led to speculation that disruptions in tumor suppressor pathways, specifically RB gene and p53 germline mutations, are important in the pathogenesis of osteogenic sarcomas. Dogs with osteosarcoma have been found to have aberrations of the p53 tumor suppressor gene (Setoguchi et al., 2001), while in laboratory animals, both DNA viruses (polyomavirus and SV-40 virus) and RNA viruses (type C retroviruses) also have been found to induce osteosarcoma (Theilen and Madewell, 1987).

Paget's Disease, characterized by varying degrees of bone dysplasia and deformity, has also been linked to osteosarcoma (Hansen et al., 2006). Pagetic osteosarcomas show evidence of alterations in the p53 pathway (Lonardo et al., 1997) and to have somatic mutations in the RB1 gene.

Another rare genetic disorder associated with an increased frequency of osteosarcomas is Rothmund-Thomson Syndrome, which is caused by mutations

in DNA unwinding proteins implicated in maintaining the stability and integrity of cellular DNA (Leonard et al., 1996).

Breed predilection. Large and giant breed dogs (>35 lb.) are particularly susceptible to osteosarcoma (90%) (Misdorp and Hart, 1979; Ru et al., 1998), while this cancer is uncommon in small and medium breeds (Theilen and Madewell, 1987). In small breed dogs, osteosarcoma represents about 45% of all skeletal neoplasms and occurs more commonly in the femur and tibia (Cooley and Waters, 1995). Breeds especially predisposed to development of osteosarcoma include Saint Bernards, Rottweilers, Great Danes, Golden Retrievers, Irish setters, Doberman Pinschers, and Labrador Retrievers (Straw, 1996). Although familial occurrences of this cancer are readily identifiable, the etiology probably involves both genetic and environmental factors acting in concert (Chun and de Lorimier, 2003).

Trauma. Prior damage at the site of tumor development may end in osteosarcoma growth (Operskalski et al., 1987). As healing and repair follow trauma, a predisposing condition is set for abnormal cell metabolism associated with rapid bone growth and for malignant osteoid to develop. Both direct malignant changes and indirect effects, such as structural changes in the stroma and connective tissue may be involved. A few varied environmental factors have been implicated as well, ranging from agricultural farming practices to fertilizers and other pesticides (Schwartzbaum et al., 1991; Kristensen et al., 1996).

Increased trauma associated with large body size and weight, especially in the major weight bearing limbs (Steverson et al., 1982) can give rise to osteosarcomas. Of the “fracture-associated sarcomas", osteosarcoma is the most common with 85% of spontaneous cases occurring in the diaphysis of the bone compared to 95% metaphyseal involvement (Straw, 1996). The femur is commonly affected, possibly due to the higher incidence of femoral fractures.

Foreign bodies (including metal implants, such as internal fixators, bullets, and bone transplants) and serious post-operative complications (especially infection) have also been implicated (Steverson et al., 1982).

Since the median time for osteosarcoma development post trauma is 5.5 years, the pathogenesis is unclear (Straw, 1996). Possible inciting events include chronic cellular repair and regeneration associated with fracture healing and osteomyelitis following trauma or infection. The overactive cell metabolism increases the likelihood of mutation and sarcoma formation. Furthermore, metal implants may corrode, releasing carcinogenic substances. Similar reactions may also occur between dissimilar or incompatible implanted metals.

B. Indeterminate Risk Factors

A number of other factors have been implicated in causing osteosarcoma, but for many of these the evidence is limited or inconsistent. As mentioned earlier, a major factor implicated in osteosarcoma development is increased growth rate, and while early studies identified height and size as notable risk factors (Fraumeni, 1967; Operskalski et al., 1987; Pui et al., 1987), more recent studies dispute these findings, reporting no correlation in humans (Gelberg et al., 1997; Buckley et al., 1998). In the early 1990s, concerns were raised about fluoridated water as a causative of osteosarcoma (Bucher et al., 1991). Since then, the issue has been extensively investigated, but the results of numerous studies indicate no correlation (McGuire et al., 1991; Young, 1991; Gelberg et al., 1995; Moss et al., 1995; Cook-Mozaffari, 1996; Bassin et al., 2006; Eyre et al., 2009).

Bone infarcts may be the inciting causes of osteosarcoma developing in unusual sites or in dogs that do not fit the usual breed, weight, or size classification (Dubielzig et al., 1981). It is thought that the medullary bone hypoxia following the infarct leads to a chronic reparative process. Although in animals the causes of bone infarcts are unknown, in humans they have been

associated with iatrogenic trauma, fat emboli, Cushing's disease, hyperviscosity, dysbaric conditions, sickle cell anemia, and alcoholism (Dubielzig et al., 1981).

IV. PATHOPHYSIOLOGY

As a primary bone cancer, osteosarcoma begins as malignant osteoid tissue in the bone from where it may spread to other parts of the body. The areas affected are the ones most active during skeletal development and predominate in weight bearing. Like most bone cancers, osteosarcoma most frequently affects the appendicular skeleton (femur, humerus, and tibia), specifically the long bones of the lower limb (Dahlin and Unni, 1986, Pool, 1990). These bones are undergoing a fast rate of growth or repair, and are, therefore, highly susceptible to giving rise to osteosarcoma. It frequently originates at or near the metaphyseal portions of the long bones, which contain bone-forming mesenchymal stem cells (Kramarova and Stiller, 1996). Osteosarcoma usually arises in the medullary cavity, penetrates the cortex, and extends into the subperiosteum, causing the formation of a large soft tissue mass contiguous to the bone.

Some osteosarcomas (extraskeletal types) arise from the soft tissues or visceral organs without the involvement of bone or periosteum (Bardet et al., 1989) (Patnaik, 1990). This neoplasm targets older animals and appears to have no breed predilection in dogs (Patnaik, 1990) or cats (Haldeman et al., 2000). It is also highly metastatic and the most common metastatic sites are the lungs and lymph nodes. Survival times range from 1 to 6 months. Extraskeletal osteosarcoma in humans represents 3-5% of all OSA, with 85% developing in the soft tissues of the extremities, especially following trauma or radiation. In humans, the incidence of metastasis is 80% and median survival time is 60 months. The periosteal (juxtacortical) osteosarcoma is a slowly progressive tumor of fibroblastic connective tissue that arises from the periosteum with

variable amounts of bone and cartilage formation (Withrow and Doige, 1980). Found predominantly in the mandible and zygomatic arch, distal tibia, distal radius, and occasionally the triceps muscle, this neoplasm is slowly progressive. A third type of osteosarcoma (chondroma rodens, multilobular osteoma), is multilobular in character and arises from the periosteal elements of the membranous bones of the skull (McCall et al., 1989). Morphologically, this tumor appears to be benign, but biologically it is metastatic. Median metastasis time is 14 months and median survival time is 21 months.

A. Histological Findings

Osteosarcoma is characterized by the presence of osteoid in the lesion, even at sites distant from bone (e.g. the lung). This osteoid formation is usually easily detected using light microscopy, although electron microscopy occasionally may be required to reveal this process. Stromal cells may be spindle-shaped and atypical, with irregularly shaped nuclei (Wittig et al., 2002).

Histologically, osteosarcoma may be distinguished into a number of variants, including conventional types (osteoblastic, chondroblastic, and fibroblastic), telangiectatic, multifocal, parosteal, and periosteal. This thesis only deals with conventional osteosarcoma, which is the most common in childhood and adolescence (Klein and Siegal, 2006).

Telangiectatic osteosarcomas are lytic neoplasms that contain large, blood-filled spaces and are seen commonly in adolescence and early adulthood (Pignatti, 1991). The parosteal type usually arises from the bone cortex, has an intermediate prognosis, and can be seen in childhood or adulthood (Klein and Siegal, 2006). It most commonly arises on the distal posterior aspect of the femur. Periosteal osteosarcoma is a low- to intermediate-grade tumor that typically arises immediately below the periosteum in children. It most frequently involves the tibia (Klein and Siegal, 2006).

B. Tumor Site

Human osteosarcoma is diagnosed most frequently in the bones comprising the knee, specifically the distal femur (31.79%), proximal tibia (15.46%). The proximal humerus is commonly affected as well, but less frequently than the bones of the leg (8.32%) (Dahlin and Unni, 1986). In dogs, osteosarcoma also shows a predilection for the appendicular skeleton (Pool, 1990). Approximately 75% of tumors occur in the long bones, most commonly involving the metaphyseal region. Moreover, the forelimbs are more frequently affected than hindlimbs. Forty percent of osteosarcoma tumors occur in the distal radius and proximal humerus, and to a lesser degree, in order of decreasing frequency, in the distal femur, proximal tibia, proximal femur, and distal tibia (Straw, 1996). Cats, on the other hand, develop osteosarcoma more frequently in the hindlimb rather than the forelimb (Turrel and Pool, 1982; Belito et al., 1987; Carpenter et al., 1987). Furthermore, feline osteosarcoma is less metastatic than the canine form. As in dogs, osteosarcoma also occurs more frequently in the appendicular than the axial skeleton (Belito et al., 1987).

On occasion, osteosarcomas may also be found to affect the axial skeleton, particularly the flat and irregular bones of the skull, especially the mandible and maxilla, as well as the ribs, scapula, vertebrae and pelvis (Patnaik et al., 1989). The type of osteosarcoma affecting the skull is usually of the multilobular type and is observed more commonly in middle-aged dogs. On the other hand, axial osteosarcoma appears to be more common in females (Ogilvie, 2001). Extraskeletal osteosarcoma is rare, but may originate in the visceral organs, gonads, skin, or mammary gland (Bardet et al., 1989).

C. Metastasis

From the long bones, the tumor often metastasizes via the circulatory or lymphatic system to other organs, preferentially the lungs (Bacci et al., 1998; Harris et al., 1998). According to the classic Multi-Institutional Osteosarcoma

Study, more than half of the patients developed metastases within six months of diagnosis (Link et al., 1991).

In the appendicular skeleton, metastasis occurs early and is locally destructive and very invasive. Osteosarcoma metastasizes primarily via hematogenous routes and rarely through the lymphatics (O'Brien et al., 1993). Metastasis occurs by primary invasion of veins and embolization to the lungs. The lung is the most common site for visceral metastases. Other metastatic sites include liver, kidneys, amputation sites, but rarely adjacent bones (Jeffree et al., 1975). Chemotherapy may alter the metastatic pattern, and evidence exists that dogs treated with cisplatin after amputation or limb-sparing surgery had more bone metastases than control animals (Berg et al., 1992). The reason for this, however, is unknown at this point.

Osteosarcoma of the axial skeleton also causes severe local invasion and destruction. However, the incidence of vascular invasion and pulmonary metastasis is less than with appendicular osteosarcoma (Patnaik et al., 1989).

IV. TREATMENT

Because osteosarcoma is a deadly form of cancer, no absolute contraindications or limits to treatment exist. And, yet, while modern medicine and research have provided the means to diagnose and evaluate, we are still limited to the mainstay combination of surgery and chemotherapy.

Pain is the most common and frequently the first symptom reported by patients with osteosarcoma (Scully et al., 2002). Decreased range of motion is also common, often in the absence of readily palpable mass. The degree of pain can vary between patients and is often determined by the proximity to

surrounding nerves or skeletal structures. In addition, bones may weaken and fracture easily.

A. Medical and Surgical Treatment

The use of a variety of effective chemotherapeutic agents expanded dramatically in the 1970s and early 1980s (Link et al., 1986). The new generations of chemotherapeutics that followed significally improved the treatment of patients with osteosarcoma through their ability to treat the micrometastatic disease afflicting approximately 80% of patients (Jaffe, 1998). Furthermore, they improved in specificity and many are less toxic than their predecessors (Hang et al., 2009). Another highlight in the treatment of osteosarcoma was the development of a surgical staging system for musculoskeletal sarcomas which allowed for an organized approach to both biopsy and definitive tumor resection of osteosarcomas and other musculoskeletal sarcomas (Enneking et al., 1980a; Enneking et al., 1980b). Amputation is no longer the only surgical solution and a number of (novel) limb-sparing and function-preserving approaches, such as autologous bone graft, allograft, prosthetic joint reconstruction, rotationplasty, and even resection of pulmonary nodules, are possible (Hang et al., 2009).

However, even though surgical techniques and chemotherapeutics have improved over the last 20 and more years, osteosarcoma therapy in itself has not substantially changed (Akiyama et al., 2008). The combination of radical resection surgery and systemic chemotherapy remain the mainstay treatment for osteosarcoma in both humans and small animals (Shenoy et al., 2008). Radiation is reserved for palliative therapy; however, no apparent benefits are discernable (Hang et al., 2009). The newest treatment for osteosarcoma involves the use of ultrasonographically guided high-intensity focused ultrasound (Li et al., 2009). High-intensity focused ultrasound ablation was reported to be a safe and feasible method of treatment of osteosarcoma, which salvages the limb, but it is clear that large-scale randomized clinical trials are necessary for confirmation.

B. Analgesic Treatment

As mentioned above, pain frequently is the first clinical symptom of osteosarcoma. Except possibly for bisphosphonates, which provide pain relief by delaying the progression of bone lesions, there are no current pharmacotherapeutical approaches to bone cancer pain that effectively relieve pain without the development of serious side effects or tolerance to the drug, particularly in terminal cancer patients (Fan et al., 2007). Cancer-induced pain usually occurs by infiltration of or exerting pressure on the pain-sensitive structures throughout the body (Porges, 1988; Regan and Peng, 2000). Cancer in the bone irritates and distends the nerve-rich periosteum resulting in ongoing pain, which is the most frequent initial symptom of bone cancer and begins as a dull, constant, throbbing and nagging pain that increases in intensity with time (Mercadante, 1997; Portenoy and Lesage, 1999) and occurs both at rest and at night (Weber et al., 2008).

Bone cancer pain results from changes occurring at both peripheral and central nervous system sites. The use of animal models of cancer pain is leading to new insights into the molecular and cellular mechanisms that contribute to the pain that follow pathophysiology (Gordon-Williams and Dickenson, 2007; Hald et al., 2009; Khasabov et al., 2007; Pacharinsak and Beitz, 2008). While we are beginning to appreciate some of the mechanisms underlying tumor-induced pain, bone pain is still treated primarily by opioid-based therapies, which are frequently accompanied by significant unwanted side effects such as tolerance and withdrawal (Miser et al., 1986; Mercadante, 1997). In addition, cancer patients on opiates typically require higher doses of opiates over time to maintain the same level of analgesia. Recent work suggests that the sensitivity of bone cancer pain to systemic morphine is lower than that of inflammatory pain (Betourne et al., 2008). One of the reasons for this is that there is a significant down-regulation of mu opioid receptors in distinct populations of dorsal root ganglia (DRG) neurons in bone cancer as compared to non-malignant inflammatory painful conditions (Yamamoto et al., 2008). As a result, patients require higher doses of morphine

to produce analgesia in bone cancer than those used in non-malignant inflammatory situations. Finally, recent work by King et al. (2007) indicates that morphine treatment accelerates sarcoma-induced bone pain, bone loss, and spontaneous fracture, suggesting that opiate treatment may result in "add-on" mechanisms of pain beyond those engaged by the sarcoma alone. Because of these side effects, there is a need to identify better treatments that can effectively reduce pain without the unwanted secondary effects of traditional pharmacological approaches.

The topic of cancer pain will be discussed in more detail in the next chapter.

V. PROGNOSIS AND SURVIVAL

In spite of the variety of effective surgical and medical therapies available today, prognoses and life expectancies post diagnosis are still guarded and short. Data collected by the SEER Program determined the overall 5-year survival rate for patients diagnosed between 1974 and 1994 to be 63%. Females had better chances of survival (70%) than males (59%).[5] The 5-year relative survival for children with bone cancer improved from 49% (1975-84), to 63% (1985-94). Advances in chemotherapy protocols have led to a 5-year survival rate of up to 68% (Hang et al., 2009), ranging from 60% to 78% (Messerschmitt et al., 2009).

The clinical prognosis for osteosarcoma is variable, but usually guarded. The present understanding of outcome and prognosis for osteosarcoma is driven by certain serum markers, clinical staging, and histologic response to chemotherapeutic agents (Hang et al., 2009). A poor prognosis is associated with high serum alkaline phosphatase (ALP) activity, tumor extension into soft

[5] http://seer.cancer.gov/publications/childhood/bone.pdf

tissue, tumor origin in a hindlimb, and the presence of pulmonary metastases (Straw, 1996). Alkaline phosphatase (ALP) is a hydrolytic enzyme present in multiple tissues including bone. Previous studies suggest that ALP activity may be within the reference interval or increased (Garzotto et al., 2000) (Kazmierski et al., 2001). However, recently ALP has been shown to be a highly sensitive and fairly specific marker in the diagnosis of osteosarcoma, since patients with an elevated ALP at diagnosis are more likely to have pulmonary metastases (Barger et al., 2005). Serum alkaline phosphatase (SAP) or calcium rarely increased (Kazmierski et al., 2001), although in humans with osteosarcoma, SAP is frequently increased. Decreased total iron binding capacity and increased ferritin concentration may be observed, and serum concentrations of zinc, chromium, and iron also may be decreased (Kazmierski et al., 2001). Dogs with osteosarcoma have decreased rates of protein synthesis, increased urinary nitrogen loss, and increased postoperative glucose flux (Mazzaferro et al., 2001). Concentration of prostaglandin E2 may be increased in dogs with osteosarcoma (Mohammed et al., 2001).

The cyclo-oxygenase 2 isoenzyme (COX-2) is expressed in a variety of malignancies in the dog as well as in humans and the potential for therapeutic and chemopreventative activity is well-established (Moalic et al., 2001), (Dickens et al., 2002), (Mohammed et al., 2004), (Mullins et al., 2004). COX-2 has been shown to be expressed in canine osteosarcoma (Mohammed et al., 2004) and expression has been associated with prognosis (Mullins et al., 2004). Thus, Cox-2 is likely to be involved in the pathogenesis of this cancer.

Another prognostic factor is the tumor itself. Low grade malignant OSA, such as fibroblastic OSA, usually has a good prognosis. Middle-aged dogs (7 to 10 years of age) generally have greater survival times than younger and older dogs (Ogilvie, 2001). A retrospective study examined the records of 331 patients with stage II osteosarcoma who had undergone surgery and chemotherapy (Kim et al., 2008). The authors found that the initial tumor size appears to be associated with a histologic response and is an important prognostic factor in

osteosarcoma. On the other hand, stage I osteosarcomas exhibit excellent prognosis, being associated with a 5-year survival of 100%, unlike stage III osteosarcomas whose 5-year survival is practically 0% (Hang et al., 2009). Interestingly, survival did not differ substantially by tumor site for osteosarcoma (Ries et al., 1999).

Perhaps most importantly, prognosis, and therefore survival, depends greatly on the patient's response to treatment. If the patient presents with local disease only, survival of 5 years or more is possible (55-65% overall), provided a combination of surgery and chemotherapy is employed (Bacci et al., 1998; Fuchs et al., 1998). The prognosis may improve dramatically and survival probability may increase significantly to 80-85%, provided chemotherapy results in a good histologic response to neoadjuvant chemotherapy (more than 90% tumor necrosis) (Rosen et al. 1982; Winkler et al., 1988; Bacci et al., 1993; Provisor et al., 1997). However, if surgery is the sole treatment, the 5-year survival rarely exceeds 15-25% surgery alone and recurrence of the osteosarcoma within 6 months is very likely (Link et al., 1986).

Approximately 20% of patients present with clinically detectable metastases, with micrometastases presumed to be present in many of the remaining patients (Messerschmitt et al., 2009). Metastatic disease dramatically decreases survival probability (20% if in lungs, less than 10% if in bones), rarely exceeding 1 to 2 years.

Thus, the long-term prognosis for both canine and human patients with osteosarcoma remains guarded. In this regard, the prognosis for patients is highly dependent on a number of factors including the size and location of the osteosarcoma, whether it is primary, metastatic or recurrent, the tumor stage, the patient's age and general health, and particularly in the case of small animals, the histological grade, surgery and mitotic index (Dimopoulou et al., 2008).

3. PAIN

I. HISTORICAL PERSPECTIVE

Almost four centuries ago, initial attempts were made to document pain perception. It was René Descartes (1596-1650), the Father of Modern Philosophy, who first formulated the theory of pain transmission occurring via a single channel from the skin to the brain (DeLeo, 2006). Descartes also was the first to postulate that the mind interacts with the body; that, in fact, even though the mind controls the body, the body had the ability to also influence the otherwise rational mind.[6] Moreover, he believed that a pain in the foot was not felt in the foot, rather it was sent through nerves dispersed through the foot up through the tibia, the thigh, the loins, the back and the neck and into the brain.[7] Descartes likened the pain mechanism to pulling on a rope to ring an alarm bell in a bell tower.

Despite its deficiencies, Descartes' specificity theory about pain perception – that pain was the direct product of a noxious stimulus activating a dedicated pain pathway and eliciting a mechanical behavioral response – remained the dominant perspective on pain until the mid-nineteen sixties, when Ronald Melzack and Patrick Wall introduced their classic Gate Control Theory (DeLeo, 2006). Prior to that momentous event, and contrary to Descartes' ideas, the German neurologist, Wilhelm Erb, proposed in 1874 that a pain signal could be generated by stimulation of any sensory receptor, provided the stimulation was intense enough (Melzack, 1996). He maintained that the pattern of stimulation rather than the receptor type was the deciding factor whether pain occurred. Twenty years later, another German neurologist, Alfred Goldscheider,

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[6] Descartes R (1649) Les passions de l'âme. Amsterdam. Reprinted in Adam and Tannery (AT), vol. XI. English translation in CSM, vol. I. English translation in Voss (1989).

[7] Descartes R (1664) L'Homme. Paris. Reprinted in AT vol. XI. Partial English translation in CSM, vol. I. Complete English translation in Hall (1972).

put forth the idea that noxious stimulation might accumulate ("summate") in the dorsal horns of the spinal cord until a threshold of accumulated stimulation was achieved which, in turn would produce a pain signal (Melzack, 1996). This idea was further studied by William K. Livingston, who, in 1943, suggested a "reverberatory" circuit in the dorsal horns as the mechanism of summation (Melzack, 1996). Finally, in 1953, Willem Noordenbos formulated the theory that a signal carried along large diameter “touch” fibers may inhibit the signal carried by the thinner "pain" fibers; the ratio between the two signals determining pain intensity (Melzack, 1996; Todd and Kucharski, 2004).

By the mid-nineteen sixties, our understanding of the central nervous system and how it communicates with the periphery (especially in response to pain) had dramatically expanded and changed accordingly: free nerve endings had been identified as pain receptors that generated electro-chemical pulses along A-δ and C "pain" fibers to the spinal cord and up the spinothalamic tract to the pain center in the thalamus (Melzack and Katz, 2003).

In 1965, Ronald Melzack and Patrick Wall introduced their classic Gate Control Theory, which revolutionized the way the scientific community looked at pain and intensely disputed Descartes’ theory (DeLeo, 2006). Until then, psychological factors had been dismissed as ‘‘reactions to pain’’ (Melzack, 1999; Jellinger, 2009). But the Gate Control Theory explained how they were an integral part of pain processing. The Gate Control Theory, essentially a neural "circuit diagram", proposes that large diameter “touch” and thin “pain” fibers meet at two places in the dorsal horn of the spinal cord the projection neurons and their "inhibitory" cells (Melzack and Katz, 2003). The signals of both fiber types converge on the projection neurons, eliciting a pain impulse (opening the gate). Inhibitory cells are programmed to inhibit this activation (closing the gate). When a noxious stimulus excites large diameter and thin cells, they simultaneously send signals to the projection neurons. Large diameter fiber signals will be activating the inhibitory cells (closing the gate), while the thin fiber signals will be impeding the inhibitory cells (opening the gate or leaving it open). Whether the gate is ultimately left open or closed (pain intensity felt) depends on the number

of large diameter fibers activated (Melzack and Wall, 1965). The Gate Control Theory also allowed for large diameter fiber signal to travel up to the brain, where they may actually trigger a signal back down to the dorsal horn to further modulate projection neuron activity and, therefore, pain intensity. This model provided a neuro-scientific explanation for the very real effects of motivation and cognition on pain. For example, the Gate Control Theory finally provided answers for such phenomena, such as spinal sensitization and central nervous system plasticity (Melzack and Katz, 2003).

Still, Descartes' reflex theory has withstood the test of time: more than 340 years later, it is still described in modern-day textbooks misleadingly as fact rather than theory and is used to justify certain analgesic strategies. However, if his theory were accurate, then the mere interruption or cutting of this pathway would alleviate all pain. Yet, experience has taught us that in real life, pain management is not as simple or straightforward as merely interrupting the nerve fiber carrying pain signals. For example, nerve damage results in the exacerbation of painful symptoms leading to chronic or neuropathic pain syndromes, as described below.

II. Epidemiology of Cancer Pain

A great variety of experimental models to study cancer pain and its management exist (Pacharinsak and Beitz, 2008), and different human and animal populations have been assessed at various stages of their illness and by various investigative tools (Brescia et al., 1992; Goblirsch et al., 2005; Breivik et al., 2009). It, therefore, is not surprising that the reported prevalence of cancer pain varies widely.

Researchers at the University of Oslo conducted a pan-European survey on cancer-related pain between 2006 and 2007 (Breivik et al., 2009). Of the 5084

adult patients questioned, 56% suffered moderate-to-severe pain at least monthly. A follow-up second-phase survey of 573 randomly selected patients identified 77% receiving prescription-only analgesics, with 41% taking strong opioids either alone or with other drugs for cancer-related pain. Of those patients who were prescribed analgesics, 63% experienced breakthrough pain, indicating inadequate pain management. In all, 69% reported pain-related difficulties with everyday activities. Of great concern was the fact that 50% believed that their quality of life was not considered a priority in their overall care by their health care professional (Breivik et al., 2009).

Chronic cancer and non-cancer pain management in North America appears equally disheartening with the pain prevalence among hospitalized patients with advanced cancer being nearly 75% (Brescia et al., 1992). In the previous chapter, I described the incidence of osteosarcoma in small animals. Unfortunately, as recently as 2007, cancer was still considered a frequently overlooked cause of pain in dogs and cats.[8]

Unable to verbally express themselves, animals communicate their pain to humans through changes in behavior (Mathews, 2000; Gaynor, 2008). Pain in neonates and young children has historically been neglected (Lee, 2002). As in animals, vocalization of the non-anesthetized human infant during surgical intervention frequently was and is still attributed to the distress of having been separated from the mother or caretaker, or to the forced restraint (Lee, 2002; Underwood, 2002). Moreover, for a long time, it was a common belief that animals were on a lower evolutionary scale than humans, and, thus, unable to experience pain (Allen, 2004). Of course, as our understanding of the physiology of pain grows in humans and animals, we can now acknowledge that animals indeed do sense pain similarly as humans do. Crane (1987) acknowledged "animal pain as a negative factor in the resolution of clinical disease or injury." Following this realization, pain management in animals followed the principles of

[8] "AAHA/AAFP pain management guidelines for dogs & cats" as put forward by The American Animal Hospital Association (AAHA) & American Association of Feline Practitioners (AAFP). Compiled by Hellyer et al. (2007).

anthropomorphism, i.e. that when in doubt, it should be considered whether the procedure would be painful in humans, and then assumed to be the same in animals. However, despite the tremendous advances in veterinary pain management over the last decade, small animals frequently are still treated inadequately for a number of painful conditions (Hellyer et al., 2007)

In adults, racial minorities, the elderly, and women frequently received inadequate analgesic therapy (Cleeland et al., 1994). As noted previously, women do exhibit lower pain thresholds than men (Berkley, 1997; Fillingim and Ness, 2000; Craft et al., 2004), and although this possibility has not been widely evaluated in the cancer pain literature, there exists documented sex bias in the treatment of cancer pain (Im and Chee, 2001; Donovan et al., 2008). In the absence of a sex or cultural bias, patients' ability to communicate the severity of their pain to their primary caretaker can affect the quality of pain management they receive (Kimberlin et al., 2004; Donovan et al., 2008). Sex differences in preferences for and response to opioid medication may determine the degree of analgesic therapy. For example, morphine is more potent in women than in men, but has a slower speed of onset and offset (Sarton et al., 2000). Consequently, women may be less likely to be prescribed strong opioids due to a greater likelihood of experiencing opioid-related side effects (Donovan et al., 2008).

In summary, this blatant disregard of suffering is rooted mainly in persisting misconceptions on pain, its assessment and consequent management (Elliott and Elliott, 1992; Schmidt et al., 1994). Social and cultural attitudes towards pain (Juarez et al., 1998; Juarez et al., 1999; Briggs, 2008), academic teachings and personal experiences or impressions (Weissman and Dahl, 1990; Sheehan et al., 1992) also serve to bias the recognition of pain. These include discrepancies between physicians' assessment of patient pain and patient self-assessment (Stephenson et al., 2009). For example, if a patient exhibits a less painful demeanor or appears in good health, in spite of experiencing moderate to severe pain, pain management is frequently inadequate (Cleeland et al., 1994).

Still, regardless of the obstacles and challenges encountered in treating chronic and especially cancer pain, a wide variety of analgesic techniques and agents are available that can provide adequate or better pain relief. Therefore, there should not be any excuses in attempts to treat pain sufficiently.

Often, and as mentioned at the beginning of this chapter, inadequate pain management occurs due to an incomplete or misguided understanding of pain, how it is elicited, transmitted and processed. Therefore, I shall now review the general pain physiology and pathways involved.

III. PAIN PHYSIOLOGY AND PATHWAYS

A. Defining Pain

The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Merskey and Bogduk, 1994). It is prudent to remember that "pain" is the subjective definition of a human emotion. Personal experiences and the anticipation of pain greatly influence a person's state of mind. Animals may not be able to "expect" pain in a similar fashion, but they can be conditioned to do so (Underwood, 2002). Fortunately, the above definition of pain was modified further by the IASP to include the conclusion that "the inability to communicate in no way negates the possibility that an individual is experiencing pain and is in need of appropriate pain relieving treatment" (Merskey and Bogduk, 1994).

When talking about "pain", it is often more appropriate to substitute the words "nociceptive," "nociceptors" or "nociception," in place of the term "pain", since these terms describe the physiological experience and eliminate the emotional aspects of pain. Nociception is the neural response to a noxious stimulus and, unlike pain, may actually occur without the conscious perception of

pain (e.g. in the unconscious or anesthetized state). Nociception occurs via specific somatic receptors, whose only function is to transmit pain information to the CNS, termed nociceptors (Merskey and Bogduk, 1994). These fire an action potential whenever exposed to stimuli, such as heat, pressure, extreme cold, chemical irritants, that threaten disruption of bodily integrity. The cell bodies of the nociceptors reside in the dorsal root ganglia or the trigeminal ganglion from where they communicate with either the spinal cord or the periphery.

Under normal conditions, the noxious stimulus must increase in intensity until a certain threshold level is overcome in order to perceive pain. This nociceptor threshold is called the pain-detection threshold. The level of maximum pain intensity tolerated is termed the pain-tolerance threshold, and it varies greatly between individuals (Sanford et al., 1986). Previously experienced pain, anticipation of pain, and medication are among those factors that influence the tolerance to pain. The pain-sensitivity range is the difference between the pain-detection and pain-tolerance thresholds.

In the periphery, the sensitivity of the nociceptor to noxious stimulus may be increased by diverse group of chemical and inflammatory mediators that control the initiation, propagation, and perception of nociception (Kitchell, 1987; Lamont et al., 2000). Tissue damage, by any cause, produces endogenous substances that act on local nociceptors. Hydrogen and potassium ions, serotonin, histamine, bradykinin, prostaglandins, leukotriens, and substance P are the most important ones (Jenkins, 1987; Kitchell, 1987; Lamont et al., 2000). These endogenous chemicals affect nociceptors, either by directly activating them, or sensitizing them to the direct effects of other algesics (substances that elicit pain), or providing an environment enhancing algesia (pain sensitivity).

Sensitization of a peripheral nociceptor increases the pain response to a given noxious stimulus, resulting in a phenomenon called hyperalgesia. Prolonged noxious stimulation affects changes in the spinal cord that facilitate pain perception. In the periphery, at the site of tissue injury, enhancement of pain perception is called primary hyperalgesia. It is mediated in part by sensitization of

primary afferent nociceptors stimulated by heat. Central enhancement, on the other hand, is called secondary hyperalgesia. Secondary hyperalgesia occurs in the uninjured tissue surrounding the site of injury and is thought to be due to sensitization in the central nervous system. Secondary hyperalgesia is characterized by hyperalgesia to mechanical, but not heat, stimuli, such as **a)** pain to light-stroking non-noxious stimuli (i.e., allodynia), and **b)** enhanced pain to punctate stimuli, such as von Frey filaments (Campbell and Meyer, 2006; Visser and Schug, 2006). It is frequently the mechanism in opioid-resistant or pathological or neuropathic pain, as well as opioid-induced hyperalgesia, conditions that respond well to the NMDA receptor antagonist, ketamine (Visser and Schug, 2006). Neuropathic pain is the result of central or peripheral nervous system adaptations, either structurally or functionally, or both, following injury (Jensen, 1996). It has recently been defined by the Neuropathic Pain Special Interest Group (Geber et al., 2009) as pain arising as direct consequence of a lesion or disease affecting the somatosensory system. It is very likely that pain syndromes in cancer patients involve both primary and secondary hyperalgesia (Campbell and Meyer, 2006).

Another term, which should be mentioned in discussions of pain and analgesia, is windup. It is the phenomenon, which calls for pre-emptive analgesia in all potentially painful situations. Wind-up occurs as a result of excessive activation of excitatory amino acids and N-methyl-D-aspartate (NMDA) receptors, as well as a build-up of inflammatory mediators within the spinal cord following painful manipulation, such as surgery (De Kock and Lavand'homme, 2007). Consequently, neurons may be damaged, resulting in a decreased pain threshold, or "windup" phenomenon, as well as central sensitization. During central sensitization, which is characteristic of chronic pain, the spinal cord neurons become more responsive to all input, even stimuli normally considered not noxious (Li et al., 1999; Herrero et al., 2000).

Prolonged continued stimulation results in physiological and structural changes in the CNS. For example, changes in expression of genes like c-fos

within the secondary (e.g., spinothalamic or spinoreticular) neurons of the dorsal horn affect the volume and type of enzymes and neuropeptides produced (Lima, 1998). One of the long-term changes that may result from this, is that the level of nerve growth factor produced increases sufficiently as to promote alteration in patterns of nerve connections in the dorsal horn of the spinal cord at or about the level of incoming pain fibers (Saab et al., 2009). This structural reorganization in the dorsal horn of the spinal cord is one factor, which contributes to an "abnormal" spread of pain. This pain is still referred to as "psychogenic" by those who, nearly 4 centuries later, continue to view pain as Descartes did (Jellinger, 2009). True psychogenic pain, however, manifests as physical pain, but is caused, increased, or prolonged by mental, emotional, or behavioral factors (Merskey, 1967; Jellinger, 2009). Merskey and Spear (1967) defined psychogenic pain as “(…) pain which is independent of peripheral stimulation or of damage to the nervous system and due to emotional factors, or else pain in which any peripheral change (e.f. muscle tension) is a consequence of emotional factors.”

The structural reorganization in the dorsal horn allows for incoming fibers from a particular area to gain access to ascending neurons and pathways that normally carry information from other distant areas and involve different levels of the spinal cord (Jensen, 1996). For example, crushing the sciatic nerve in rats initially produces hyperalgesia in the skin innervated by the sciatic nerve. However, within days, hyperalgesia develops well beyond this area, and even includes structures supplied by the femoral nerve (Van Remoortere et al., 2007). Similar observations were reported following sciatic nerve stimulation in a rat model of gastrocnemius-soleus muscle inflammation (Hoheisal et al., 1994; Mense et al., 1997).

Clinically, pain may be classified by its location in the body, its type, and its duration. Peripheral pain, as the term implies, originates in the periphery. It is said to be either somatic or visceral based on its site of origin (Crane, 1987): somatic pain originates from the skin, muscles or bones, while visceral pain is

related to the internal organs and viscera and associated primarily with serosal irritation (Crane, 1987; Kitchell, 1987; Wright, 1987). When pain originates in the peripheral nerves, following trauma, for example, it is called neurogenic pain. Central pain arises from a pathology or dysfunction of the CNS due to, for example, spinal cord injury, multiple sclerosis, stroke and epilepsy (Boivie, 1996).

Somatic, or superficial pain, can be subdivided into first and second pain. First pain is associated with tissue injury and characterized by a sharp, stabbing, well-localized pain sensation. The delayed pain sensation, which is dull, burning and diffuse in character, that follows is the second pain (Kitchell, 1987; Sager, 1993). Pain may also be defined as being acute (nociceptive) or chronic. It may be felt as a sudden sharp pang, as in acute trauma, or as prolonged dull ache, as often is the case in chronic joint disorders. Acute pain caused by trauma, surgery or infection, is characterized by a well-defined and abrupt onset, as well as physical signs of autonomic nervous system activity (Kitchell, 1987). Analgesic therapy and healing of the injured tissue usually alleviate this type of pain. In human medicine, when pain persists for longer than six months, it is considered chronic. Its onset is not well defined, there is no autonomic nervous system response and it is often unresponsive to pharmacological treatment (Meyr and Saffran, 2008).

B. Nociceptive Pathways

The perception of pain requires two types of nerve fibers. Thinly myelinated A-δ fibers deliver the rapid early component of noxious sensation (phasic, or first, pain), while the unmyelinated C fibers convey the slower, delayed, protracted element (tonic, or second, pain) (Sager, 1993; Ørstavik et al., 2003). Some A-δ fibers are referred to as high-threshold mechanoreceptors because they respond to moderately intense noxious mechanical and not to chemical or thermal stimuli (Kitchell, 1987). These A-δ fibers are responsible for the first pain sensation. In contrast, C (or polymodal) nociceptors respond to various activators including high-intensity mechanical stimulation, as well as chemical and thermal stimuli

(Ørstavik et al., 2003). Both A-δ and C type fibers innervate the deep somatic structures such as skeletal muscle, joints, bones and fascia, although their distribution density is variable (Lamont et al., 2000). Both fiber types appear to play a role in joint-nociception, but noxious stimulation of muscle and tendons that results in diffuse deep pain seems to suggest innervation by C fibers.

The first step in nociception involves the conversion of the noxious stimulus by specialized free nerve endings of afferent neurons (nociceptors) into electric nerve impulses (Sager, 1993; Lamont et al., 2000). This will only occur, however, if the intensity of stimulation reaches a certain threshold. Tissue damage is followed by an inflammatory response (release of chemical mediators, such as histamines and bradykinin) and a drop in pH, to which small non-myelinated C fibers are sensitive (Millan, 1999). Furthermore, transient receptor potential vanilloid1 (TRPV1) receptors are implicated in increased levels of pain sensitivity seen during inflammation (Marx, 2004; Gunthorpe and Szallasi, 2008) and in association with osteosarcoma (Menéndez et al., 2006). C fibers may be activated by high level mechanical and thermal stimulation, but they are primarily chemical sensors (Fitzgerald and Woolf, 1984). After stimulation, they generate an electrical impulse, which travels along the nerve to the dorsal horn of the spinal cord. C fiber activity may be up-regulated peripherally by serotonin and inflammatory mediators, such as prostaglandins, thromboxane, and leucotrienes, in a process called peripheral sensitization (Menétrey and Besson, 1982). Further sensitization of peripheral C fibers may be due to the release of substance P, cytokines, chemokines and a variety of other agents (Jimenez-Andrade et al., 2009). Both, peripheral and central sensitization, are important mechanisms in chronic pain conditions.

At the dorsal horn, C fibers release the excitatory neurotransmitters glutamate, aspartate, calcitonin gene related peptide (CGRP), as well as nitric oxide (Jensen, 1996; Besson, 1999). Glutamate is one of the major neurotransmitters involved in the transmission of pain information, and it acts on

a variety of glutamate receptors in the dorsal horn neuron, particularly the NMDA receptor (Besson, 1999; De Kock and Lavand'homme, 2007).

The dorsal horn also contains the "pain gate" described by Melzack and Wall (1965), where signals transmitted by the large A-β nerve fibers can alter the sensitivity of the post-synaptic cells to the painful stimuli arriving via C and A-δ delta fibers. Also located at this level are enkephalin-producing descending fibers from the brain stem and intrinsic enkephalin producing neurons, which interact with both pre-synaptic and post-synaptic cells to inhibit impulse transmission or attenuate the pain signal before it is passed rostrally (Hökfelt et al., 1977). This modulating descending system connects the periaqueductal gray (PAG) and higher brain centers with the spinal cord. There are a number of neurotransmitters associated with this descending system and this includes endogenous opioid polypeptides, which bind to specific (opioid) receptors on both the first order and second order nociceptive neurons. When an endogenous exogenous opioid binds to an opioid receptor, noxious stimulus induced neurotransmitter release from the primary afferent nerve terminals may be reduced or inhibited leading to a reduction or inhibition of action potentials from second order neurons in the pain pathway.

Increased frequency of stimulation at the synapse in the dorsal horn causes the post-synaptic electrical discharge to become more prolonged, with consequent increase in the severity of the pain (Staud et al., 2007). In essence, this is "windup", which was described earlier. The mechanism of transmitting the electrical signal occurs by means of cation (primarily sodium ion) channels (Elliott and Elliott, 1999; Waxman et al., 1999; Catterall, 2000; Wood et al., 2004). Whenever sodium rushes into the neuron from the extracellular space, potassium flows out, and the action potential is generated and propagated along the nerve toward the spinal cord. The sodium is then quickly pumped out of the cell (while potassium enters the cell again), priming it to discharge again when another stimulation occurs (Catterall, 2000).

The action potential is transmitted to the primary nociceptive neurons in the dorsal root ganglia in the CNS along their associated afferent axons. When it arrives at the spinal cord, it makes its first synapse in the dorsal horn. The cells in the dorsal horn are divided into physiologically distinct layers called laminae. Laminae I, II, V, and X contain nociceptive neurones (Willis and Chung, 1987). Both A-δ and C fibers terminate in the superficial dorsal horn. A-δ fibers form synapses in Laminae I and V, C fibers connect with neurons in lamina II, and A-β fibers connect with lamina I, III, & V (Lamont et al., 2000). Initial integration and modulation of nociceptive input occurs at this point. From here, nociceptive fibers (first order neurons) project to second order neurons and traverse the surface of the cord to enter the dorsal horn of the spinal gray matter. In this multilayered region, a complex form of local processing takes place, which largely determines the output to the ascending pathways and higher CNS centers. Projection neurons are essential for processing of nociceptive information, and eventually convey dorsal horn nociceptive input to supraspinal centers via one of several ascending pathways (Willis and Chung, 1987).

C. Somatosensory Pathways

The most prominent nociceptive pathway in the spinal cord is the spinothalamic tract. After crossing the midline, it ascends on the opposite side of the spinal cord to terminate in the lateral and medial thalamus, which acts as a relay point for sensory information going to the cerebral cortex (Kitchell, 1987). It allows rapid processing as well as sensory discrimination that provides the precise information needed to deal quickly with a painful stimulus (Mense, 2004). The spinothalamic tract is concerned with rapid transmission of nociceptive information about the onset of trauma, its precise location, and its severity. Thus, the sharp, shooting readily localized pain (phasic pain) that arises very rapidly following injury is perceived through the lateral ascending pathways (Mense, 2004).

In addition to the spinothalamic tract, several supraspinal pain modulating loops exist, which are able to modulate pain intensity. Most of the other descending anatomical pathways are primarily inhibitory, but some may facilitate pain perception (Millan, 2002). During the fight-and-flight response, when safety is more important than pain perception, it is the brain that is responsible for sorting out whether to pay attention to the painful area or to ignore it. The reticular formation of the brain stem is involved in either facilitation or inhibition of pain perception under the influence of cortico-reticular signals. By last count, there are at least five spinoreticular (or reticulospinal) loops on each side of the body, passing information in both directions, and they may be inhibitory or facilitatory (Willis and Westlund, 1997). These loops connect the spinal cord to the brainstem, specifically the dorsolateral pontine tegmentum, the rostral ventral medulla, the dorsal medulla, the caudal medulla, and the lateral hypothalamus (Willis and Westlund, 1997).

Another pathway participating in nociception is the spinohypothalamic pathway. It is located deeper in the spinal cord and contains thin fibers that form multiple synapses as they ascend (Zhang et al., 1999). However, it does not synapse in the reticular formation. It carries information of emotional significance from the periphery directly to the hypothalamus (Zhang et al., 1999). Impulses conveyed by this and the spinoreticular tract ascend more slowly than those carried by the myelinated and monosynaptic tracts. There is no rapid perception or discrimination of pain, but rather a diffuse, unpleasant aching sensation may be felt for some time after the injury has occurred. The descending system powerfully inhibits the dorsal horn and modulates input passing through it. It, thus, can rapidly inhibit the lateral system. As a result, phasic pain is of brief duration. Finally, the dorsal column pathway transmits visceral nociception to the thalamus (as well as somatic touch and position sense). Now this is known to be so (Hirshberg et al., 1996).

Most of the axons belonging to inhibitory secondary neurons cross the midline and synapse in the thalamus (Willis and Westlund, 1997). From there,

information about pain is further transmitted rostrally for interpretation and modulation. Because the thalamus is an important structure in mediating emotions and feelings, it makes sense that pain is usually an emotional experience. With acute pain, the emotion is usually anxiety (Meyr and Steinberg, 2008), while chronic pain is frequently linked to depression (Rottmann et al., 2009; Teh et al., 2009).

D. Intrinsic Pain-Inhibiting Mechanisms

In addition to the descending inhibitory pathways a number of intrinsic pain-inhibiting mechanisms exist that provide analgesia in painful situations. A number of external events (e.g. trauma from an injury, stress from temporary exposure to cold, or treatment with acupuncture needles) can lead to a decreased sensitivity to pain. This extraordinary intrinsic inhibition of pain is termed stress-induced and stimulus-produced analgesia.

There are situations when pain is not the first priority, when there is a need for self-protective pain reduction, such as during fight-or flight. The anesthesiologist Henry K. Beecher, who has been called the father of the “prospective, double-blind, placebo-controlled clinical trial”, was an early pioneer in the study of the relationship between subjective psychological states and objective drug responses. In 1946, he published his observations of pain perception in soldiers wounded during battles in World War II:

> "Three-quarters of badly wounded men, although they have received no morphine for hours... have so little pain that they do not want pain relief medication, even though the questions raised remind them that such is available for the asking. This is a puzzling thing and perhaps justifies a little speculation."

Upon arrival at the field hospitals, the majority of soldiers refused morphine. They were relieved to be alive and did not regard their wounds as terribly important (Beecher, 1946). Ten years later, Beecher studied a group of

civilians with surgical incisions comparable in size to the wounds he had seen on the battlefield. The civilians felt that the operation was a catastrophic and traumatizing event in their lives and overall had a rather pessimistic outlook. He noted that the majority of these patients demanded morphine (Beecher, 1956). Similar events occur, when an athlete (e.g. a football player) fractures a fibula and continues to run on the leg. While he most certainly is experiencing pain, at the moment, the goal is more important, and the pain is reduced or ignored. Stress-induced analgesia has been confirmed in animal models of stress due stressed with electric shocks or forced swimming in cold water (Liebeskind et al., 1976; Watkins and Mayer, 1982).

Electrical stimulation of certain brain structures, such as the PAG region of the midbrain in the unanesthetized animal, results in significant analgesia in the periphery (Reynolds, 1969). Opiate and stimulus-produced analgesia are mediated by a common neural mechanism (Basbaum et al., 1976; Basbaum et al., 1977), although non-opioid mechanisms exist also (Sternberg and Liebeskind, 1995).

Acupuncture is another modality, which produces analgesia by stimulation (Melzack and Wall, 1984). In 1974, Linzer and Van Atta were able to reduce the response of feline thalamic neurons to painful stimulation of the skin by applying acupuncture at remote areas of the body. The concept of acupuncture analgesia will be dealt with in detail in Chapter 4.

Finally, transcutaneous electrical nerve stimulation (TENS) has found clinical application for pain control following surgery (Cooperman et al., 1977; Ali et al., 1981), in back injuries, with shingles, and osteoarthritis (Jensen et al., 1991). Electrodes are placed near the painful area, and stimulus intensity is increased until either muscle twitching, or pain, or both, is noticed. Then the intensity is reduced until relief is felt. Transcutaneous nerve stimulation and electro-acupuncture are similar in working by excitation of the same types of nociceptors, and in producing analgesia that outlasts the stimulus.

IV. PATHOPHYSIOLOGY OF BONE CANCER PAIN

I described the pathophysiology specific to osteosarcoma-induced pain in the previous chapter. At this point I would like to delve further into the pain associated with bone cancers. The pathophysiology of cancer pain is complex, but it helps to describe it in terms of nociceptive and neuropathic pain. Earlier in this chapter, I classified pain and described the differences between the two types of pain. For nociceptive pain to occur, intact nociceptors and neurons are required. Neuropathic pain, on the other hand, is due to structural or functional changes in the nervous system. It frequently arises following chemotherapy, in part due to disruption of tubulin function, followed by the release of cytokines, resulting in degeneration of sensory neurons and sensitization of primary nociceptive afferents (Mantyh et al., 2002). Tissue fibrosis with nerve compression and microvascular obstruction of the nerve following radiotherapy has also been observed (Mantyh et al., 2002). Neuropathic pain syndromes will be described in more detail later in this chapter.

The first and most obvious cause of nociceptive pain is cancer surgery. As with other surgical interventions, the pain is acute and somatic and due to direct tissue trauma, as well as related inflammatory changes (Meyr and Saffran, 2008). However, persistent somatic pain is also diagnosed when neoplastic growth infiltrates the musculo-skeletal system (Viganó et al., 1998; Walsh, 2005), or exerts physical pressure on bone or surrounding tissues, as well as compression of the peripheral nerve or vasculature (Mantyh et al., 2002). In addition to these mechanical causes of cancer pain, several biochemical, molecular, and neurobiologic mechanisms may be implicated in bone pain:

a) With advanced disease, the bone loses mechanical strength and is subject to osteolysis, pathological fracture, and microfractures. Mechanical distortion of the periosteum and interaction between osteoblasts and osteoclasts may be a major source of pain (Delaney et al., 2008; Colvin and Fallon, 2008).

b) Proteolytic enzymes produced by tumor cells can damage sensory and sympathetic nerve fibers, causing neuropathic pain (Mantyh et al., 2002).

c) The tumor itself may release chemical substances directly involved in nociception**.** For example, blocking tumor-associated mediators, including TNFα (Wacnik et al., 2005b) endothelin (Wacnik et al., 2001) calcitonin gene-related peptide (CGRP) (Wacnik et al., 2005a), nerve growth factor (McMahon, 1996; Sevcik et al., 2005) or cyclooxygenase 2 (COX-2) (Sabino et al., 2002) significantly reduces, but does not completely inhibit, tumor-induced nociception.

Thus, excitation and sensitization of local nociceptors occurs via a local and systemic inflammatory response, a communal effort mounted by the cancer cells themselves, inflammatory cells and the immediate vasculature. In addition to the chemical mediators mentioned earlier (prostaglandins, bradykinin, cytokines, nerve growth factor, etc.), several other factors such as ATP, endothelin-1 (Asham et al., 2001; Baamonde et al., 2004) and vascular endothelial growth factor are released, which directly affect primary afferent pain fibers (Wacnik et al., 2001; Wacnik et al., 2005b). Moreover, tumors release protons, causing local acidosis, with similar effects of sensitization (Mantyh et al., 2002).

After action potential generation and transmission, the nociceptive information is processed in the spinal cord and then relayed via a number of ascending somatosensory pathways, which include the spinothalamic and spinocervicothalamic tracts, to higher centers of the brain, as described above.

V. Pain Due to Cancer Treatment

The neoplasm itself (including its products or the changes it causes in the surrounding tissues) is not always the sole cause of cancer pain. Indeed, the

very therapies employed to treat the cancer frequently may be a source of pain (Porges, 1988).

Painful neuropathy is an increasingly common problem facing the cancer patient with significant impact on quality of life (Blumenthal, 2009). The condition most commonly starts immediately after cancer surgery (Steegers et al., 2008), or it may arise at a later point (van Wilgen et al., 2004). Neuropathic pain presents as an ongoing, shooting, or burning ache in the areas innervated by the nerve(s) damaged during surgery (Willis and Westlund, 1997; Steegers et al., 2008; Vadivelu et al., 2008; Dualé et al., 2009).

A result of a primary lesion or dysfunction in the nervous system (Bridges and Thompson, 2001), it is characterized by a paradoxical loss of sensation occurring alongside hypersensitivity at the site of injury (Jensen et al., 2009). There may be one or a combination of the following signs in the painful area (Abdi et al., 1998; Park et al., 2008; Steegers et al., 2008; Vadivelu et al., 2008; Authier et al., 2009; Dualé et al., 2009): paresthesia (burning, prickling, itching, tingling, or numbness with no apparent physical cause), dysesthesia (unpleasant abnormal sensation), hypoesthesia (diminished sensation), or allodynia (pain produced by a non-noxious stimulus, such as light touch).

Post-mastectomy pain syndrome (PMPS), a neuropathic pain syndrome, is a common after-effect of mastectomy surgery (Smith et al., 1999; Vadivelu et al., 2008). An aching or burning pain across the site of the surgery, or extending along the distribution of the intercostobrachial nerve which is usually damaged during the surgery, is frequently reported (Vadivelu et al., 2008). Like many other forms of neuropathic pain, PMPS is relatively resistant to treatment once it has manifested itself and negatively affects the quality of life (Eisenberg et al., 2007). However, the syndrome can be prevented by perioperative administration of an NMDA receptor antagonist, such as amantadine (Eisenberg et al., 2007) or ketamine (Visser and Schug, 2006; Sarrau et al., 2007). When the intercostal nerves are damaged during thoracotomy for removal of lung cancer, a post-thoracotomy pain syndrome can develop, which presents as burning, aching, or

piercing pain along the distribution of the nerves (Steegers et al., 2008; Dualé et al., 2009). Interestingly, up to half the chronic pain after thoracic surgery is not associated with a neuropathic component (Steegers et al., 2008), which would explain why perioperative ketamine does not prevent chronic neuropathic pain after thoracotomy (Dualé et al., 2009). Neuropathic pain as occurs also as a result of the toxic effects of various chemotherapy drugs, such as vincristine, cisplatin, and paclitaxel, on the distal peripheral nerves (Park et al., 2008; Authier et al., 2009). Paresthesia and dysesthesia are common signs, and, in the case of paclitaxel, acute myalgia (Dina et al., 2001).

Opioids can be another and quite peculiar contributing cause of cancer-associated pain. King et al. (2007) showed that morphine treatment accelerates sarcoma-induced bone pain, bone loss, and spontaneous fracture, suggesting that opiate treatment may result in "add-on" mechanisms of pain beyond those caused by sarcoma alone. This phenomenon of morphine-induced hyperalgesia is regulated by the TRPV1 receptor (Vardanyan et al., 2009). The TRPV1 receptor is a molecular sensor of noxious heat that responds to multiple forms of noxious stimuli and plays an important role in the development of inflammation-induced hyperalgesia, as mentioned earlier. Interestingly, morphine may increase the number of subpleural pulmonary metastases when given subsequent to tumor cell injection (Simon and Arbo, 1986). In this rat model of bone cancer (using Walker 256 carcinosarcoma cells), naloxone blocked this increase in metastases, as did the partial μ-receptor, pentazocine, confirming an opioid mechanism. However, there does not appear to be any clinical data do support this finding. It appears unlikely that up- or down-regulation of MOR mRNA levels is the mechanism involved (Gach et al., 2009). A recent study showed that several opioid antagonists, including naloxone, produced up-regulation of MOR gene expression but no antiproliferative effects on MCF-7 breast cancer cells, neither in the presence or absence of beta-estradiol (Gach, et al., 2009).

Finally, radiation therapy seldom causes pain. However, in a few isolated cases discomfort at the site of irradiation may be experienced years after

receiving radiation therapy (van Wilgen et al., 2004). Unlike the cases with surgery and chemotherapy, this pain is both nociceptive and neuropathic (Epstein et al., 2009), meaning the pain probably arises from actual tissue damage, which probably leads to peripheral sensitization. This implies that usual nociceptive pain therapies, such as use of opioids, either alone or in combination with non-steroidal anti-inflammatory drugs (NSAIDs) can be effective.

The treatment of neuropathic pain differs significantly from nociceptive pain, and reducing primary hyperalgesia by usual means is inefficient. The pain signal of neuropathic pain does not arise from normal nociceptive terminals, but rather at the site of a neuroma, or along the course of a damaged nerve, or even in the dorsal root ganglion (Devor et al., 1992). Therefore, therapy is this case aims to reduce the ectopic discharge in the nociceptive neuron. Local anesthetic agents, such as lidocaine may block the signals, either topically (Gammaitoni and Davis, 2002) or injected near the neuroma. Tricyclic antidepressants are another class of drugs often useful in the management of neuropathic pain (Reisner, 2003). It may be that blocking of sodium channels, rather than the reuptake of norepinephrine and serotonin, is their analgesic mechanism in neuropathic pain patients (Pancrazio et al., 1998). Tramadol is a synthetic narcotic-like drug with analgesic properties similar to opioids with fewer gastrointestinal side effects (Grond et al., 1999). However, unlike opioid drugs, Tramadol exerts its analgesic properties by inhibiting the reuptake of norepinephrine and serotonin from presynaptic neurons (Pypendop and Ilkew, 2008).

Gabapentin has achieved widespread use in the management of neuropathic pain (Rowbotham et al., 1998). Synthesized as an analogue of the neurotransmitter gamma-aminobutyric acid (GABA), it was originally developed for the treatment of epilepsy (Baillie and Power, 2006). Unfortunately, the exact mechanism by which gabapentin improves neuropathic pain is not known at this point. Gabapentin is not believed to act on the same brain receptors as GABA, but is thought to exert its analgesic effect by binding to the α2δ subunits (1 and 2) of voltage-gated N-type calcium ion channels in the CNS (Hendrich et al., 2008).

In the absence of serious side effects and few drug interactions, gabapentin is an attractive alternative in the treatment of neuropathic pain (Rowbotham et al., 1998).

VI. Opioid Analgesic Therapy for Cancer Pain

Opioid analgesics have long been recognized as among the most effective treatments for pain (Hamilton and Baskett, 2000). The 17th century English physician Thomas Sydenham wrote in 1686:

> "Among the remedies which it has pleased Almighty God to give man to relieve his suffering, none is so universal and so efficacious as opium."

More than 400 years later, opioids remain the mainstay analgesics in treating both nociceptive and neuropathic pain. Opioids control pain by binding to specific receptors in the CNS, thereby raising the pain threshold (Jenkins, 1987). Opiate receptors have been identified by homogenizing brain tissues and incubating them with labeled opiates or antagonists (Kuhar et al., 1973; Terenius, 1973; Lowney et al., 1974; Snyder, 1975). High densities of opiate receptors were found in the periaqueductal and periventricular gray matter. This was later confirmed with autoradiographs of brain sections (Goodman et al., 1980). More neurochemical mechanisms of pain inhibition were identified by reversal of opioid analgesia with the specific opioid antagonist, naloxone (Liu and Wittbrodt, 2002; Brainin-Mattos et al., 2006; Goodman et al., 2007).

Five types of opiate receptors have been described that account for the various physiologic aspects of narcotic activity (Yaksh, 1984). However, only the mu (μ), kappa (κ), delta (δ), and epsilon (ε) receptors are involved in producing analgesia (Pascoe, 2000). Mu receptors are sensitive to morphine, its derivatives and morphine-like drugs (Kieffer et al., 1995), kappa to dynorphin (Goldstein,

1988) and butorphanol, delta to enkephalins, and epsilon to endorphins (Yaksh, 1984). Three genes, referred as to MOR, DOR and KOR, have been cloned, which encode mu, delta and kappa receptors, respectively (Kieffer et al., 1995). This discovery has contributed greatly to understanding of opioid receptors, their role in pain and analgesia, as well as various side effects (Buesa et al., 2008; Cheng et al., 2008).

Activation of pain-modulating neurons leads to the inhibition of transmission from primary afferent nociceptors to dorsal horn sensory projection cells. The mechanism by which they achieve this, is by diminishing both the likelihood of neurotransmitter release and action potential generation in post-synaptic nociceptive neurons (Haigler, 1978). In this manner, opioids directly affect pain transmission in the spinal cord without altering nerve conduction or afferent nerve fiber sensitivity. They also alter the reaction to pain by affecting both the sensory-discriminative and motivational-affective components of it (Carr, 1984; Pedersen and Blackburn-Munro, 2006). In fact, the second effect may be the most important, since patients often report that, with morphine treatment, the pain is still present but they do not mind it as much. Morphine can also bring about a significant placebo-effect (Benedetti et al., 2007). However, it has also been reported experimentally and clinically that exposure to opiate can elicit paradoxical pain (opiate-induced hyperalgesia) in regions of the body unrelated to the initial pain complaint. Several mechanisms have been suggested to be responsible for opiate-induced hyperalgesia including sensitization of peripheral nociceptors, enhanced production/release of glutamate and neuropeptides in the spinal cord, protein kinase C gamma-induced signaling, and/or enhanced descending facilitation of nociceptive pathways from the rostral ventromedial medulla (reviewed by White and Wilson, 2010)

VII. NON-STEROIDAL ANTI-INFLAMMATORY DRUG THERAPY FOR CANCER PAIN

Because nociceptive pain, by definition, begins by stimulation of nociceptors, interventions that inhibit the normal pain pathway may be useful in treating it. For mild to moderate cancer pain, the first intervention occurs by means of nonsteroidal anti-inflammatory drugs (NSAIDs).

These drugs inhibit the synthesis of prostaglandins at one or more points in the complex biosynthesis pathway, typically by blocking the action of cyclooxygenase (COX) enzyme (Marnett and Kalgutkar, 1999; Kay-Mugford, 2000), which catalyzes the first two reactions of the arachidonic acid pathway in the formation of eicosanoids, such as prostanoids, prostacyclin (PGI2), and prostaglandin E2 (PGE2) as well as the thromboxanes, which are all involved in various homeostatic functions depending on the tissue, organ, or cell involved. Since the early 1990s it has been known that two forms of COX exist – the homeostatic COX-1 (Vane et al., 1998; Marnett and Kalgutkar, 1999; Ballou et al., 2000; Kay-Mugford, 2000), and the inducible COX-2, which is primarily associated with inflammation (Siebert et al, 1997; Marnett and Kalgutkar, 1999; Jones and Budsberg, 2000; Turini and DuBois, 2002). However, more recently, a third form of cyclooxygenase, COX-3, has been suggested, which is expressed predominantly in the cerebral cortex and heart and has been implicated in a primary central mechanism by which NSAIDs produce analgesia and reduce fever (Chandrasekharan et al., 2002).

In addition to infection or injury, inflammation may also occur at tumor sites (Lin and Karin, 2007). Cyclooxygenase-2 is expressed in a variety of malignancies in the dog as well as in humans (Moalic et al., 2001; Dickens et al., 2002; Mohammed et al., 2004; Mullins et al., 2004). Because of their relatively favorable side effect profile, COX-2 inhibitors are attractive choices to diminish the inflammatory component of mild to moderate cancer pain (Jenkins and Bruera, 1999). Furthermore, COX-2-inhibiting NSAIDs, such as meloxicam, exhibited antineoplastic effects in vitro (Naruse et al., 2006; Wolfesberger et al.,

2006). Thus, COX-2 is a very interesting target for cancer therapy and pain management.

VIII. ANTICANCER THERAPY FOR PAIN RELIEF

Most commonly, surgery is a treatment modality for the cancer itself, rather than cancer pain (as outlined in the previous chapter). Yet, there are some circumstances when surgery may be used for pain managment. For example, patients with impending fractures of the long bones quick pain reduction and reduced orthopedic disability may be achieved by the insertion of an endoprosthesis (Mercadante, 1997).

Chemotherapy may be used to reduce pain in symptomatic patients whose symptoms cannot be easily managed with less toxic and less expensive therapy. Patients with metastatic prostate cancer, for example, may have no survival advantage from chemotherapy with mitoxantrone plus prednisone, but may have a significant improvement in pain control and overall quality of life from those drugs (Osoba et al., 1999). Pallliative chemotherapy for other tumor types may show similar improvement (Ellison and Chevlen, 2002).

Radiation therapy, such as bone irradiation, is often used to treat painful metastases, with a response rate of 60% to 80% and onset of pain relief often within the week (Serafini et al., 1998; Yarnold et al., 1999). Single dose irradiation was comparable to multiple fraction irradiations in pain relief and side effects with equivalency lasting at least a year (Yarnold et al., 1999).

Therapeutic radionuclides are not part of the “usual” therapy. Strontium 89 chloride and Samarium 153 EDTMP (ethylenediaminetetramethylphosphonate) are the two FDA-approved radionuclides found useful in the management of painful bone metastases (Porter et al., 1993; Quilty et al., 1994; Serafini et al., 1998). Following injection into the bloodstream, the radioactive agent

concentrates in the bone, particularly in bone metastases. Its radiation energy (beta irradiation) travels only a few millimeters through the bone with minimal toxicity. Within a week, patients may begin to observe a decrease in their pain (Serafini et al., 1998). Not only do therapeutic radionuclides reduce the pain of bone metastases, but when used as an adjuvant to external beam irradiation, they also reduce the likelihood of the emergence of new painful sites (Porter et al., 1993).

Like therapeutic radionuclides, bisphosphonates are not analgesics, but are remarkably useful in the management of pain due to bone metastases. Bisphosphonates bind to the hydroxyapatite crystals in bone, where they are thought to inhibit the activity of osteoclasts, thus preserving the integrity of the bone (Berenson et al., 1996; Hortobagyi et al., 1996; Hillner et al., 2000), as well as reducing the risk of pathological fractures (Lipton et al., 2002; Saad et al., 2002).

IX. Complementary and Alternative Analgesic Therapies

Complementary therapies – adjunctive, effective techniques that treat symptoms associated with cancer and its mainstream treatment – play an increasingly important role in controlling symptoms, especially pain and nausea, associated with cancer or cancer treatments (Beuth and Schierholz, 2007; Cassileth et al., 2007; Lu et al., 2008). Other therapies commonly employed and found of value include meditation to reduce stress and herbal teas to relieve nausea (Cassileth and Deng, 2004). However, more in-depth, controlled and standardized studies need to be conducted to better understand and evaluate complementary treatments and their therapeutic value in cancer medicine.

From a clinical standpoint, such complementary therapies as needling acupuncture and electroacupuncture are advocated in both human and

veterinary medicine as adjunct therapies to treat the adverse effects of cancer treatment, or to contribute to multimodal pain management (Staud and Price, 2006). The mechanisms by which acupuncture and electro-acupuncture exert their analgesic effects are several. For one, it is possible that acupuncture works through potentiation and modulation of a highly organized and somatotopic network of endogenous opioids that links expectation, attention and the body (Liu, 2008).

Electroacupuncture also produces an immediate, segmental analgesia of short duration that is not mediated by endogenous opioids, but may be explained by the gate control theory (Ernst and Lee, 1987). Acupuncture can raise the levels of endogenous opioids in the cerebrospinal fluid (Zhang et al., 1980), as well as in the serum (Xi et al., 1983). Furthermore, in a rat model of bone cancer pain, electroacupuncture attenuated bone-cancer-induced hyperalgesia by inhibition of spinal preprodynorphin (Zhang et al., 2008) and spinal interleukin-1 β (Zhang et al., 2007c). Naloxone has repeatedly reversed the analgesic effects of acupuncture, supporting an endogenous opioid mechanism (Pomeranz and Chiu, 1976; Mayer et al., 1977).

As mentioned earlier, both canine and human osteosarcomas express COX-2 (Mullins et al., 2004; Masi et al., 2007). Many neoplasms are also pro-inflammatory (Sabino et al., 2002; Lin and Karin, 2007). Electroacupuncture significantly decreases COX-2 expression in different models of pain and hypersensitivity (Lau et al., 2008) and inflammation (Lee et al., 2005; Lee et al., 2006).

Thus, acupuncture and electroacupuncture are serious contenders in providing an alternative to conventional (cancer) pain management. This topic will be discussed more in the next section.

4. ELECTROACUPUNCTURE

I. HISTORICAL PERSPECTIVE

Acupuncture is a popular complementary and alternative medical modality that is used to treat a wide variety of medical conditions both in humans and animals (Chan et al., 2001; Lee et al., 2005).

Even though China and Eastern Asia are considered the places of origin of acupuncture, it is important to note that similar modalities developed independently in other locales and cultures (Gori and Firenzuoli, 2007): For example, the placement of needles to treat various ailments was also employed in Peru and by the shamans of the American Southwest. Both the Bantu tribes of South Africa and the Eskimo tribes in the Far North still utilize scratching and stimulation of the skin at specific body points to induce healing. Similarly, certain native tribes in Brazil use tiny blow darts shot at specific areas of the body to induce healing. Furthermore, the Ebers papyrus, which dates back to 1550 BC, describes medical practices in ancient Egypt and discusses the concept of energy flowing along channels. Arabic physicians used ear cauterizations, a precursor to the current treatment of auricular acupuncture, to treat sciatica. The placement of acupuncture needles in the ears and hands, rather than the body or the feet, likely developed due to the physicians' inability to touch women during treatment, a long-time social taboo in many cultures (Guo, 1995).

Interestingly, the earliest evidence of acupuncture does not come from China, but rather dates back to prehistoric Europe (Gori and Firenzuoli, 2007). In 1991, the mummified remains of Ötzi the Iceman were discovered in the

Tyrolean Alps.[9] In addition to carrying medicinal mushrooms and other herbs, the 5,300 year old mummy displayed “a series of short, dark, parallel lines” tattooed on his back, right knee and left ankle, where modern day X-rays detected signs of arthritis.[10]

Still, the earliest surviving records on the use of acupuncture were written by Chinese physicians between 480 and 220 BC. However, there is evidence to suggest that it may have been practiced in China as early as 4,500 years ago (Wu, 1996). Following the spread of acupuncture knowledge from China to Western Europe by merchants and Jesuit missionaries from the 17th to the 19th century, the interest of Western physicians was mixed (Kaptchuk, 1997). Physicians of the East India Company were among the earliest to advocate these new Eastern medical techniques in the late 17th and early 18th centuries, but faced outright hostility from medical colleagues at local universities on the continent and in the United Kingdom (Stollberg, 2007). In spite of such noteworthy proponents of acupuncture as William Osler, Franklin Bache (great-grandson of Benjamin Franklin), Louis Berlioz (father of the composer Hector Berlioz), John Churchill, and John Elliotson (Kaptchuk, 1997), by the beginning of the 20th century, acupuncture had all but vanished from medical practice in Europe.

Interest in acupuncture saw a new surge before World War II in France and after the War in Western Germany (Gleditsch 2001). But acupuncture, as it is known today, originated in China in the 1940s. It was born out of necessity when only a handful of doctors trained in Western medicine were available to care for a vast rural population requiring medical care (Ulett et al., 1998).

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[9] Australian Broadcasting Corporation (2005) Scientists look at the iceman, nicknamed Oetzi. Accessed online on 11/23/09 at http://www.abc.net.au/news/indepth/featureitems/s1305469.htm

[10] Ice Age Acupuncture? Study of Mummified Body Raises Questions about Practice’s Origin. Acupuncture Today (2000) 1:6. Accessed online on 11/23/09 at http://www.acupuncturetoday.com/pdf_out/AcupunctureToday.com-Ice-Age-Acupuncture-1259013768.pdf

Chairman Mao Tse-Tung solved this daunting problem by having thousands of practitioners trained in Chinese folk medicine, which had formerly been outlawed, and by sending them all over the country.

The first scientific reports on surgical procedures being carried out under “acupuncture anesthesia” used in surgical operations surfaced in China in the 1960s (Ulett et al., 1998). This extraordinary phenomenon has been studied ever since, but even though acupuncture can induce an analgesic effect, no scientific evidence exists that confirms any true anesthetic effect (Han, 1995). In fact, Han (1995) considers the term “acupuncture anesthesia” a misnomer, and proposes the term ‘acupuncture-assisted anesthesia’ instead, as the analgesic effects of acupuncture alone are ineffective for most surgical procedures. However, when used in conjunction with other analgesic drugs as part of multimodal pain management, acupuncture analgesia is potentiated (Cao, 1997; Zhu et al., 1997a; Zhu et al., 1997b; Zhang et al., 2004b). In fact, this potentiated analgesic effect may be sufficient for surgery (Han, 1997; Wu, 2005).

Real sustained interest in acupuncture, however, did not take place until 1971, when New York Times reporter, James Reston, a member of President Nixon’s press detail on his visit to China, had to undergo an emergency appendectomy. Following the surgery, he received acupuncture treatment for postoperative pains and discomfort (Reston, 1971). His account of this new "miracle cure” that "could even substitute for anaesthesia" stirred interest in the general public and was the starting point of serious scientific investigations into acupuncture and Traditional Oriental Medicine (TOM) (Ulett et al., 1998).

One year later, the NIH sponsored an acupuncture study for the first time. The study distinctly differentiated acupuncture from hypnosis (Ulett, 1983). In 1981 and 1991, the American Medical Association Council and the National Council Against Health Fraud, respectively, declared that acupuncture had no scientific basis. Yet, the interest of both the public and the research community persisted, and in 1997, the NIH advocated the usefulness of acupuncture as a

therapeutic complementary intervention in various painful conditions (Ulett et al., 1998).

Veterinary acupuncture has been practiced in China for at least 2,000 years (Haltrecht, 1999). As public interest in TOM therapies grew in the West, interest in veterinary acupuncture also has risen dramatically over the past 25 to 30 years. Acupuncture has been found effective in treating many various disease and pain conditions in horses, cattle, dogs, cats, and birds (Bossut et al., 1984; Janssens et al., 1988; Steiss et al., 1989; Xie et al., 1996; Looney, 2000; Xie et al., 2001; Iwa et al., 2005; Xie et al., 2005).

Many geriatric patients have generalized pain, arthritis, hind end weakness, and chronic diseases that hinder their quality of life. Some of these patients are also too weak to undergo conventional therapy and thus require an alternative to conventional treatment that is safe and effective. Acupuncture is one such treatment modality (Xie, 2004).

II. APPLICATION OF ACUPUNCTURE

Acupuncture is a popular complementary and alternative medical modality that is used to treat a wide variety of medical conditions both in humans and animals (Chan et al., 2001; Lee et al., 2005).

In the absence of any significant side effects, only a few contraindications to acupuncture treatment exist (Chung et al., 2003): a) depth of needle insertion is determined by the location of the acupuncture point or health status of the patient. For example, needles placed on the thorax cannot be inserted to the same depth as needles over muscles of the limb; b) needles should never be inserted directly into a tumor or open wound, c) pregnancy precludes use of certain acupuncture points, especially those around the lumbar and lower

abdominal regions; d) needling at CV-8 acupuncture point is contraindicated, and the point is reserved for moxibustion only; e) electroacupuncture should not be used in patients with seizures or cardiac pacemakers.

A. Systemic Effects or Disease Conditions

The most thoroughly explored and understood acupuncture mechanism is acupuncture analgesia, and, as such, will serve to explain in this chapter how acupuncture and electroacupuncture exert their many effects. Descriptions of other mechanisms, such as immune modulation, will be noted where appropriate.

Pain. While early reports on the positive effects of acupuncture on pain and certain disease processes were primarily anecdotal, recent controlled studies suggest that acupuncture can indeed relieve pain, reduce inflammation and successfully treat certain medical conditions (Napadow et al., 2008). For instance, recent systematic reviews of the effect of acupuncture on chronic headaches concluded that acupuncture could be a valuable non-pharmacological treatment tool (Linde et al., 2009), and that needling acupuncture is superior to sham acupuncture and medication therapy in improving headache intensity, frequency, and response rate (Sun and Gan, 2008).

There is also strong evidence that acupuncture can be a useful supplement to other forms of conventional therapy for nonspecific lower back pain (Yuan et al., 2008) and for the treatment of postoperative pain (Sun et al., 2008). Further support for the effectiveness of acupuncture for back pain in dogs with thoracolumbar intervertebral disk disease showed that electroacupuncture combined with standard Western medical treatment was effective and resulted in shorter time to recover than did use of Western treatment alone (Hayashi et al., 2007). Similarly, recent research suggests that acupuncture is an important therapy for treating neuropathic pain in dogs and cats (Mathews, 2008) and humans (Taguchi, 2008).

Regular acupuncture treatment results in gradual elevation of the pain threshold in both humans and animals, which implies a delayed effect, as well as a long-lasting analgesic effect after acupuncture treatment has ceased (Chiang et al., 1973; Pomeranz and Chiu, 1976; Mayer et al., 1977; Han et al., 1983; Cui et al., 2005). For example, acupuncture manipulation at the LI-4 acupuncture point resulted in a peak increase of the pain threshold 20–40 min after needle insertion, which lasted for over 30 min after withdrawal of the needle. This analgesic effect was inhibited by injection of 2% procaine into LI-4 just prior to acupuncture (Chiang et al., 1973; Han et al., 1983; Farber et al., 1997).

In spite of the ever-growing body of evidence validating the analgesic effectiveness of acupuncture, many systematic reviews of clinical acupuncture studies fail to find convincing evidence that acupuncture relieves pain (Ee et al., 2008; Tough et al., 2008; Wang et al., 2008a; Madsen et al., 2009). For instance, Pittler and Ernst (2008) recently concluded that “the evidence is not fully convincing for most complementary and alternative medicine modalities in relieving neuropathic or neuralgic pain”.

The conflicting observations of acupuncture effects reported in the literature are likely due to the large variation in experimental conditions, experimental design, use of adequate controls and the patient population or the animal pain models used. Thus, many questions should be raised when reviewing acupuncture data in the literature. These include the following: Which pain model was used? Did the investigators employ manual or electroacupuncture, and, in the case of electroacupuncture, what were the electrical current parameters? Which acupuncture point was studied and how deep were the needles inserted? Further differences relate to methodological problems and issues, including limited sample sizes, lack of randomization, inappropriate control groups, and discounting the concept of Eastern Medicine philosophy.

Cardiovascular effects. A direct link exists between acupuncture and the autonomic nervous system. Vasomotor symptoms have a significant physiologic correlation to increased sympathetic nervous system activation (Freedman, 2005). Acupuncture causes increases in heart rate variability and increased cardiac parasympathetic modulation in healthy subjects (Haker, 2000). A systemic evaluation of the role of complementary and alternative therapies in cardiac rehabilitation concluded that "some complementary and alternative medicine therapies hold promise for patients in cardiac rehabilitation. Further research is essential, however, in all areas of complementary and alternative medicine to confirm its usefulness as an adjunct to cardiac rehabilitation." Arhur e tloa., 2006). Another systematic review of the effect of acupuncture on cardiac arrhythmias concluded that "acupuncture seems to be effective in treating several cardiac arrhythmias, but the limited methodologic quality of the studies necessitates better-controlled clinical trials" (VanWormer et al., 2008). Acupoint stimulation affects blood pressure receptors and, thus, has the ability to modulate blood pressure (Iwa et al., 2005; Zhang et al., 2009). In that regard, Zhang and coworkers (2009) showed that acupuncture can lower systolic blood pressure, but not diastolic blood pressure. Electroacupuncture treatment after a stroke may assist with early rehabilitation and decrease time in a nursing home by 50% (Johansson et al., 1993; Magnusson et al., 1994).

Immune-modulating effects. Stimulation of the parasympathetic vagus nerve has been shown to have a direct effect on the inflammatory immune response by decreasing serum levels of pro-inflammatory cytokines (Tracey, 2007). Acupuncture can also activate T-cell lymphocytes and increase the number of white blood cells for the treatment of immunodeficiency (Yuan and Zhou, 1993). The anti-inflammatory of acupuncture occur via a reflexive central inhibition of the innate immune system (Kavoussi and Ross, 2007; Cabioğlu and Cetin, 2008), resulting in a decrease in proinflammatory (TNFα, IL-1β, IL-6, and IL-18) and an increase in anti-inflammatory (IL-10) cytokines (Kavoussi and Ross, 2007; Cabioğlu and Cetin, 2008). Furthermore, decreases in cortisol (Han et al., 2004)

and the release of ACTH, implying the involvement of the hypothalamo–pituitary corticotrope axis (Pan et al., 1996).

Gastrointestinal Disorders. Electrical stimulation of the Zusanli (ST-36) acupuncture point has been used effectively to inhibit experimentally-induced gastrointestinal disorders in dogs (Jin et al., 1992). Electroacupuncture also was found effective clinically in the treatment of gastrointestinal disorders (Li et al., 1992).

Psychological Effects. Electroacupuncture has been used successfully for the treatment of depression (Han, 1986; Loti et al., 1990), posttraumatic stress disorder (Ulett and Nichols, 1996), and anxiety (Ulett, 1996).

Addiction. Electroacupuncture in the ear has been used successfully to treat addiction (Wen and Cheung, 1973), but a placebo effect has been noted when non-stimulated needles were used (Ter Reit et al., 1990; Wells et al., 1995; Otto et al., 1998). Han et al. (1994) successfully used transcutaneous stimulation of body acupuncture points in the treatment of heroin addicts. The alternating high (100 Hz) and low (2 Hz) frequency stimulation produced the most significant improvement of withdrawal symptoms.

B. Placebo Effect

The involvement of psychological factors as the underlying mechanism for acupuncture-induced analgesia is a possibility (Price et al., 1984). Dr. Ted Kaptchuk and his team of researchers at Harvard University have devoted much of their research to studying the effects of placebo and expectation. When they compared the analgesic effects of manual acupuncture to electroacupuncture and placebo in healthy subjects, verum acupuncture treatment, but not placebo,

lowered pain ratings in response to calibrated noxious thermal stimuli (Kong et al., 2005). Based on their observation that some of the study participants responded only to electroacupuncture and others only to manual acupuncture, the authors suggest that acupuncture analgesia may be dependent on both subject and mode (Kong et al., 2005).

A great variety of psychological or physical stressors are able to stimulate the hypothalamo–pituitary axis. This results in the release of ACTH, which modulates a number of physiological responses. Electroacupuncture enhances plasma ACTH and up-regulates the expression of fos in the hypothalamic–pituitary corticotrope axis (Pan et al., 1996). Both responses can be blocked by deprivation of nociceptive primary afferent input using capsaicin. Interestingly, ATCH release and fos expression due to immobilization-induced stress were not changed by capsaicin treatment, which suggests that electroacupuncture may depend on the physiological afferent signals elicited in the somatosensory pathway (Pan et al., 1997).

However, the physiological mechanisms of acupuncture analgesia outweigh any psychological factors involved (Ezzo et al., 2000; Lundeberg and Stener-Victorin, 2002; Lee and Ernst, 2005). In fact, several studies demonstrated the superior effects of acupuncture when compared to placebo in patients (Lewith and Machin, 1983; Richardson and Vincent, 1986; Pariente et al., 2005) and in healthy volunteers (Lee and Ernst, 1989; Ulett, 1989; Bausell et al., 2005).

C. Differences in response to Acupuncture

The analgesic effect of acupuncture is subject to individual differences.

In addition to inherited genetic factors (Lee et al., 2002; Chae et al., 2006), physical health may be an important factor affecting an individual's response to acupuncture. The analgesic effects of acupuncture were studied in rats with and without inflammation (Sekido et al., 2003). Some of the normal rats responded to

acupuncture treatment, while others did not. However, all of the rats with inflammation responded to treatment and manifested acupuncture analgesia.

II. WHAT IS ACUPUNCTURE?

A. Meridian Theory

Traditionally, acupuncture was practiced in the context of interactive diagnostic procedures based on complex philosophical laws (Vieth, 1949). One of the key Eastern Medicine philosophical concepts underlying the effectiveness of acupuncture centers on stimulating the Qi (life force or energy), which is thought to flow through the body (Wax and White, 2000). In healthy individuals the Qi is balanced between two major forces, Yin and Yang. Additionally, 12 organ systems were associated with the five elements fire, earth, metal, wood, and water (Vieth, 1949).

The Qi flows along energy pathways or meridians, which are precisely mapped channels that connect to each other and to the various body organs (Mayer, 2000). Modern acupuncture recognizes 14 meridians: liver (LIV), gallbladder (GB), heart (HT), small intestine (SI), pericardium (PC), triple heater (TH), spleen (SP), stomach (ST), lung (LU), large intestine (LI), kidney (KID), bladder (BL), governing vessel (GV), and conception vessel (CV) (Schwartz, 1996). In accordance with the meridian theory, pain is the result of a disease-induced blockade of the meridians. Acupuncture stimulation of specific points along these meridians is thought to remove the blockade and restore the proper energy balance.

Once considered purely a philosophical concept, scientific evidence suggests that meridians are real and consist of blood vessels (traditional), sheets of connective tissue (Langevin et al., 2002; Langevin and Yandow, 2002), or

nerves (Li et al., 2004). Since there is no consensus regarding the exact anatomical structures constituting meridians, the energy channels may be viewed as a functional modality that involves a summation of multiple physiological functions and organ systems (Zhou, 2008).

B. Acupuncture Points

In the ancient text, “The Yellow Emperor's Classic of Internal Medicine”, it is stated that “On these meridians there are 365 acupuncture points, one for each day of the year” (Veith, 1994).

In actuality, there are 361 acupuncture points located on 14 meridians and their branches throughout the body. Acupuncture points can be divided into four types, according to their location (Liu et al., 1975; Gunn, 1978; Looney, 2000): motor points are found where a nerve enters muscle, midlines points are located on dorsal and ventral midlines, some points reside over nerves or nerve plexuses, and points at muscle-tendon junction are associated with the Golgi bodies. Acupuncture points exist in bilateral symmetry and may differ electrically from surrounding tissue (Brown et al., 1974).

Of the 361 acupuncture points located on the skin of the human body, 323 exhibited innervation by peripheral nerves (Zhou et al., 1979). Interestingly, early studies did not find afferent fibers innervating the skin to be important structures in mediating acupuncture signals (Chiang et al., 1973; Shen et al., 1973; Han et al., 1983). Moreover, recent findings of a pilot study employing immunohistochemistry reported a significantly decreased number and density of subcutaneous nerve structures in acupuncture points compared with non-acupuncture points in human (Wick et al., 2007). Conversely, when the distribution of afferent nerve endings in relation to acupuncture points was studied in the rat hindlimb, the receptive fields for both A and C fiber afferents were concentrated either at the sites of acupuncture points or along the meridian channels (Li et al., 2004). In addition, the majority of deep sensory receptors

were located at acupuncture points in muscle tissue (Li et al., 2004). Therefore, it is quite possible that acupuncture points may be excitable muscle-skin-nerve complexes with a high density of nerve endings.

It should be noted that acupuncture points are relatively specific. In this regard both manipulation and site of needling contribute significantly to the elevation of pressure pain threshold following acupuncture (Zasiawski et al., 2003). Stimulation of classical acupuncture points elicits significantly higher activation of the hypothalamus and primary somatosensory-motor cortex, as well as deactivation of the rostral segment of anterior cingulate cortex, than stimulation of non-acupuncture points (Zhang et al., 2004). The activation of the bilateral visual cortex located in the occipital lobes was shown by fMRI to cause bilateral stimulation of vision-related acupuncture points located in the lateral aspect of the foot (Siedentopf et al., 2002). Stimulation of non-acupuncture points, on the other hand, causes no activation in the occipital lobes (Wu et al., 2002). To be fair, while there is evidence for acupoint specificity, a recent critical review of methodological problems in fMRI studies of acupuncture focusing on visual and auditory cortex activation highlights the fact that some acupuncture-fMRI studies have not adopted all methodological standards applied to most other fMRI studies (Beissner and Henke, 2009). These authors conclude that activation of the visual and/or auditory cortex reported by some fMRI studies were probably not a direct result of acupuncture stimulation per se but rather attributable to one or more methodological problems that include the choice of baseline, interpretation of deactivations, attention control and implications of different group statistics.

The segments of the body comprise dermatomes, myotomes, sclerotomes and viscerotomes, in which the same level of innervation and sensory input enter the spinal dorsal horn (Campbell, 1999; Bogduk, 2002). When acupuncture points are located in the same segmental innervation regions (e.g. ST-36) versus remote spinal segments (e.g. LI-4), acupuncture analgesia is more effective (Wu et al., 1974; Dai et al., 2001). These anti-nociceptive effects are even more

pronounced when the acupuncture points selected reside along the same nerve innervating the receptive field of the source of pain (Wu et al., 1974). This evidence may point towards a functional specificity of acupuncture points (Bing et al., 1991a), even though several acupuncture points distant from the pain sites also are efficient for relieving pain (Wu et al., 1974; Bing et al., 1990; Zhu et al., 2004a).

C. Acupuncture

Acupuncture may be defined as the stimulation of specific locations on the body (acupoints or acupuncture points) using needles (acupuncture), touch (acupressure), or heat (moxibustion).

The focus of this chapter will be on the two acupuncture techniques used most frequently in clinical situations: a) manual needling, the most common technique, involves insertion of a sterile filiform acupuncture needle into the acupuncture point, followed by twisting of the needle to achieve stimulation; and b) electroacupuncture requires insertion of the needle into an acupuncture point just as manual acupuncture, but in addition a stimulating current is delivered through the needle. Electroacupuncture may also involve the placement of a surface electrode on the skin over the acupuncture point instead of needle insertion (Wang et al., 1922; as cited in Ulett et al., 1998). This electroacupuncture-like surface stimulation is delivered to the skin over acupuncture points at low-frequency and high-intensity until strong muscular contractions are evoked and the pain threshold increases (Andersson and Holmgren, 1975). This mode of electrical stimulation, however, may not be confused with transcutaneous electrical nerve stimulation (TENS), where the surface electrodes are placed over the painful area rather than at a specific acupuncture point (Melzack and Wall, 1984). Transcutaneous electrical nerve stimulation is carried out at the site of pain using high-frequency, low-intensity stimulation (Lundeberg, 1984; Chan and Tsang, 1987).

Successful acupuncture effects are dependent on and determined by needle manipulation, resulting in a feeling frequently described by patients as soreness, numbness, heaviness and distension in the deep tissue beneath the acupuncture point (Pomeranz, 1989; Haker and Lundeberg, 1990; Hui et al., 2005). The acupuncturist, in turn, feels a tissue pull, or 'grabbing' of the needle, and increased resistance to manipulation (Langevin et al., 2001; Kong et al., 2005; MacPherson and Asghar, 2006). This sensation is termed de-Qi, and it occurs via central pathways: damage to the anterior commissure of the spinal cord is accompanied by pain and temperature sensation deficits, and patients experience no or reduced acupuncture effects and de-Qi (Cao, 2002). Clinically, the efficacy of acupuncture analgesia is highly dependent upon the de-Qi (Wang et al., 1985; Pomeranz, 1989; Haker and Lundeberg, 1990; Hui et al., 2005).

An early investigation of de-Qi consisted of a needle inserted deeply into the LI-4 or ST-36 acupuncture point for stimulation and EMG recording (Shen et al., 1973). The EMG recordings from the muscle beneath the acupuncture point could be positively correlated to the intensity of the subjective sensation derived from acupuncture manipulation (numbness, soreness, etc.) needle grasp, the de-Qi. Injection of procaine into the muscle beneath the acupuncture point blocks both sensations (Shen et al., 1973). Although de-Qi appears to originate mainly from muscle stimulated by acupuncture, it may also stem from other deep tissue due to activation of polymodal receptors (Kawakita et al., 2002).

The needle grasp characteristic for de-Qi has been suggested to be due to a mechanical coupling that occurs between the needle and connective tissue when the tissue is wound around the needle during manipulation (Langevin et al., 2001; Langevin et al., 2002). This winding, or rotating (twisting or twirling) of the needle, may produce a mechanical signal in the tissue and excite local nerve fibers and structures (Gunn, 1978; Langevin et al., 2001; Langevin et al., 2002). In addition, needling produces changes in circulation, temperature, or chemical effects (Peng and Greenfield, 1990).

For example, when acupuncture was administered at ST-36, a significant analgesic effect was observed, as well as an enhanced degranulation of mast cells (Zhang et al., 2007a). Pharmacological destruction of the mast cells weakened the analgesic effect, suggesting an important role of mast cells in connective tissue in acupuncture analgesia.

The types of afferent fibers activated, as well as individual differences in sensitization, depend on different acupuncture modes and manipulations (Langevin and Yandow, 2002). Thus, the acupuncture needle should be considered a physical sensory stimulus, which activates a diverse array of receptors depending on its intensity, frequency, duration and interval between stimulations (Linde et al., 2009). Manual acupuncture is able to activate A-β, A-δ, and C afferent fibers (Okada et al., 1996; Zhu et al., 2004a). Electroacupuncture has the added potential of being able to produce stimulation intense enough to excite the small, myelinated sensory type II A-β (Wu et al., 1974; Levine et al., 1976; Toda and Ichioka, 1978; Lu et al., 1979; Pomeranz and Paley, 1979; Chung et al., 1984; Toda, 2002) and type III A-δ (Wu et al., 1974; Kawakita and Funakoshi, 1982; Leung et al., 2005) afferents to produce analgesia.

Acupuncture at LI-4, ST-36, and Yanlingquan (GB-34) acupuncture points is capable of modulating the descending inhibitory pathways (Wu et al., 1999; Li et al., 2000; Liu et al., 2000; Yan et al., 2005). The specific structures activated include the periaqueductal gray (PAG) and the nucleus raphae magnus (NRM) in the midbrain, dorsomedial nucleus of the thalamus, hypothalamus, nucleus accumbens, and primary somatosensory-motor cortex are activated by acupuncture. Interestingly, multiple limbic regions involved in modulating pain emotion, such as the rostral part of the anterior cingulate cortex, the amygdala and the hippocampal complex, are deactivated by the same stimulation (Wu et al., 1999; Price, 2000; Gao et al., 2004; Lei et al., 2004).

The ability of acupuncture to modulate pain processing implies that it likely is capable of also modulating central homeostasis to produce analgesia. In

addition, this would explain how acupuncture regulates the balance of Yin and Yang according to the ancient meridian theory.

D. Manual Acupuncture versus Electroacupuncture

Manual acupuncture at the LI-4 acupuncture point can increase the pain threshold in the healthy individual (Chiang et al., 1973). Injection of procaine to block the cutaneous branches of the radial nerve innervating the skin at LI-4 failed to have an effect on the acupuncture-induced analgesia. However, when the deep branches of the ulnar nerve and the median nerve innervating the muscles at LI-4 were blocked using procaine acupuncture analgesia was completely abolished (Chiang et al., 1973). This suggests that excitation of afferent fibers originating primarily in the muscle underlies the effects of acupuncture, at least at LI-4.

As mentioned above, the de-Qi feeling essential for analgesia results from strong mechanical stimulation of the muscles by repetitive manipulation of acupuncture needles inserted into acupuncture points. Different types of afferents are activated depending on stimulation intensity and duration. Gentle manipulation will excite A fibers, while more rigorous manipulation of the acupuncture needles causes sufficient damage to underlying muscle and other deep tissues, resulting in the release of proinflammatory mediators, such as histamine, bradykinin, PGE2, 5-HT and ATP, which, in turn, excite local nociceptors (Boucher et al., 2000; Meyer et al., 2005). It is, therefore, quite possible that C-type fibers are involved in manual acupuncture-induced analgesia (Wei et al., 1973; Wei et al., 1978). Acupuncture analgesia produced by stimulation of ST-36 was completely abolished in rats following capsaicin treatment of both sciatic nerves to selectively block A-δ and C fibers. This mechanism of C fiber mediation of acupuncture analgesia is similar to that evoked by so-called diffuse noxious inhibitory control, which is mediated by A-δ and C fibers (Okada et al., 1996; Zhu et al., 2004a).

Electroacupuncture. The use of electrically stimulated needles was first reported in the 1960s (Sheng and Chang, 1960; Schwarz, 1966). This electroacupuncture provided the statistically most significant surgical analgesia (Parwatikar et al., 1979; Leong and Chernow, 1988) when compared to traditional acupuncture manual stimulation procedures.

Electroacupuncture-induced analgesia occurs by both pre- and post-synaptic inhibition in the spinal neurons. Strong electroacupuncture stimulation at Huantiao (GB-30) and GB-34 or ST-36 acupuncture points induced significant enlargement of antidromic C-waves of the sural nerve (Fung and Chan, 1976; Li et al., 1993). These findings suggest that electroacupuncture can cause an enhanced depolarization in the presynaptic terminals of primary C afferents, which results in inhibition of the release of neurotransmitters from these terminals. Electroacupuncture stimulation at ST-36 also produces inhibitory post-synaptic potentials, as well as a long-lasting membrane hyperpolarization in nociceptive neurons of the spinal dorsal horn (Wu et al., 1978).

Electroacupuncture has produced bilateral analgesic effects in human subjects and animal models (Han et al., 1983; Takakura et al., 1995; Kim et al., 2000; Lao et al., 2004). However, the common consensus is that only high intensity stimulation can produce lasting analgesia (Romita et al., 1997). There has been some contention about which types of afferents (specifically whether C fibers) actually mediate electroacupuncture analgesia (Hu, 1979; Liu et al., 1986; Liu et al., 1990).

Scientific evidence from several animal and human studies suggests that high intensity electrical stimulation produces analgesia by exciting type II A-β afferents (Wu et al., 1974; Levine et al., 1976; Toda and Ichioka, 1978; Lu et al., 1979; Pomeranz and Paley, 1979; Chung et al., 1984; Toda, 2002). More potent analgesic effects are seen, however, following stimulation of type III A-δ afferents (Wu et al., 1974; Kawakita and Funakoshi, 1982; Leung et al., 2005). Electroacupuncture activates all types of A fibers, and induces strong nociceptive

inhibition of spinal dorsal horn neurons (Wu et al., 1974; Pomeranz and Paley, 1979).

Brief administration of low-frequency electroacupuncture to the Yinbai (SP-1) and Dadun (LR-1) acupuncture points on the left lower extremities caused significant increase of the warm thresholds of both medial calves and significant reduction of the acute thermal pain threshold at the ipsilateral calf during electrical stimulation, but not during pre-acupuncture or post-acupuncture periods (Leung et al., 2005). This implies that even though electroacupuncture most likely produced its analgesic effects by stimulating A-δ fibers, it had an inhibitory effect on the C fibers. The possibility of C fiber involvement in electroacupuncture analgesia was tested also by experimentally induced degeneration of primary afferent C fibers using 50 mg/kg of neonatal capsaicin (Zhu et al., 1990b). Electroacupuncture analgesia was significantly reduced in capsaicin-treated animals compared to controls. Analgesia persisted following blockade of A-β and A-δ afferent fibers, suggesting the involvement of C fibers in electroacupuncture analgesia.

Clinical observations seem to support the notion of C fiber involvement in electroacupuncture analgesia in animal experiments (Chung et al., 1984; Bing et al., 1990). Conversely, degeneration of C afferent fibers by neonatal capsaicin treatment causes loss of TRPV-1 expressing neurons in the DRG (Kissin, 2008). After capsaicin treatment, injection of formalin into the hindpaw results in a significantly reduced level of fos expression in the dorsal horn (Uchida et al., 2003). However, following electroacupuncture administration, fos expression was unaffected by capsaicin treatment. Thus, it is possible that electroacupuncture induces the expression of fos in dorsal horn neurons via capsaicin-insensitive afferents, such as A-δ fibers, rather than C afferents (Pan et al., 1997).

These conflicting results are most likely due to the great variety of experimental conditions represented in the body of literature. Apart from the experimental models and acupuncture points used, electrical current parameters

(duration, biphasic or monophasic pulses), frequencies and intensities vary tremendously from study to study.

Electroacupuncture also produces an immediate, segmental analgesia of short duration that is not mediated by endogenous opioids, but instead complies with the gate control theory of Melzack and Wall (Ernst and Lee, 1987). It is of interest that one recent theory indicates that acupuncture works through potentiation and modulation of a highly organized and somatotopic network of endogenous opioids that links expectation, attention and body schema (Liu, 2008).

Thus, when comparing the peripheral afferent mechanisms of acupuncture analgesia, manual acupuncture and electroacupuncture are comparable – even though electroacupuncture appears to act predominantly via A-β and A-δ afferents, and manual acupuncture stimulates all afferents, especially C fibers. Since acupuncture and electroacupuncture induce their anti-nociceptive effects by several mechanisms resulting in a multimodal analgesic effect, which can compete with pharmaceutical analgesic drugs. However, the combination of (electro-) acupuncture with common analgesic drugs (Han, 1995) or the simultaneous use of both acupuncture techniques would produce more potent analgesia than either alone (Kim et al., 2000).

III. Neurophysiologic Theory of (Electro-) Acupuncture

Acupuncture analgesia is essentially a manifestation of integrative processes at different levels of the CNS between the afferent impulses from the pain regions and impulses from acupuncture points (Zhao, 2008). Consequently, the physiological, biochemical, and brain mechanisms involved in acupuncture analgesia have been the focus of numerous investigations (Chang, 1973; Chang, 1980; Han and Terenius, 1982; Han, 1986; Vincent and Richardson, 1986; Chung, 1989; Han, 1989; Takeshige, 1989; Ulett, 1989; Sims, 1997; Ulett et al.,

1998; Mayer, 2000; Pomeranz, 2001; Cao, 2002; Le Bars and Willer, 2002; Han, 2003; Staud and Price, 2006; Wang et al., 2008b). The neurologic model conceived 30 years ago, still represents the most widely accepted physiologic mechanism attributed to acupuncture stimulation (Pomeranz 1978; Cheng and Pomeranz 1981).

Impulses generated by acupuncture stimulation of acupuncture points travel from the peripheral nerves to the dorsal horn of the spinal cord. From there, they ascend along one of six potential pathways including the anterolateral and spinothalamic tracts to the hypothalamus, whose primary ascent occurs via the ventrolateral funiculus (VLF) (Chiang et al., 1975; Li et al., 2007).

In the brain, impulse processing occurs in the nuclei of the central endogenous descending inhibitory system. These nuclei include the NRM, the PAG, the locus coeruleus, the arcuate nucleus, the preoptic area, the nucleus submedius, the anterior pretectal nucleus, the habenular nucleus, the nucleus accumbens, the caudate nucleus, the septal area, and the amygdale (Fields et al., 2005). When activated, these structures mediate acupuncture analgesia. Only the habenular nucleus and locus coeruleus antagonize it (Bing et al., 1991b; Takeshige et al., 1991; Yang et al., 1992; Lee and Beitz, 1993; Takeshige et al., 1993; Wu et al., 1995; Guo et al., 1996; Hui et al., 2005; Yan et al., 2005).

The use of functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) as means to identify the central effects of acupuncture in the human brain is becoming increasingly popular (Wu et al., 1999; Hui et al., 2000; Hsieh et al., 2001; Kong et al., 2002; Wu et al., 2002; Zhang et al., 2003a; Zhang et al., 2003b; Fang et al., 2004; Zhang et al., 2004; Yan et al., 2005). Both fMRI and PET scans have confirmed many of the early research findings and hypotheses regarding acupuncture mechanisms by demonstrating signal changes in those CNS structures thought to represent regions of the central endogenous descending inhibitory system. These signal changes are considered to represent increased or decreased neural activity, and have been positively correlated with acupuncture needle insertion (Cho et al.

1998; Biella et al. 2001; Gareus et al. 2002; Kong et al. 2002; Wu et al. 2002; Hui et al. 2005; Pariente et al. 2005; Yan et al. 2005).

A. Neural pathways and brain structures

The phenomenon of acupuncture analgesia is frequently attributed to the gate control theory (Ernst and Lee, 1987), but in truth, the pathways of acupuncture analgesia form complex interactions with the pain pathways in the CNS and associated neurotransmitters (Cao, 2002). The main ascending and descending pathways of pain are well-documented (Millan, 1999; Millan, 2002) and have been described in the previous chapter.

The endogenous descending inhibitory system in the CNS plays a vital role in processing acupuncture analgesia (Shen et al., 1974; Shen et al., 1975; Shen et al., 1978; Hu et al., 1980). The NRM and its adjacent structures are of particular importance (Du and Zhao, 1975; Du and Zhao, 1976). Impulses generated by acupuncture stimulation of acupuncture points ascend primarily via the VLF (Chiang et al., 1975; Li et al., 2007). In the brain, impulse processing occurs in the nuclei of the central endogenous descending inhibitory system. These nuclei include the rostral ventromedial medulla (Fields et al., 2005), the NRM (Liu et al., 1986; Liu et al., 1990), the periaqueductal gray (Sheng et al., 2000; de Medeiros et al., 2003; Guo et al., 2004), the locus coeruleus, the arcuate nucleus, the preoptic area, the nucleus submedius, the anterior pretectal nucleus, the habenular nucleus, the nucleus accumbens, the caudate nucleus (Zhang et al., 1978; He and Xu, 1981; He et al., 1985), the septal area, and the amygdale (Fields et al., 2005). When activated, all these structures, except the habenular nucleus and the locus coeruleus, mediate acupuncture analgesia (Bing et al., 1991b; Takeshige et al., 1991; Yang et al., 1992; Lee and Beitz, 1993; Takeshige et al., 1993; Wu et al., 1995; Guo et al., 1996; Hui et al., 2005; Yan et al., 2005).

B. Opioid-mediated Analgesia

The last three decades have seen a surge of renewed scientific interest in acupuncture and electroacupuncture. Numerous studies strived to identify central and peripheral effects of electroacupuncture and acupuncture (Nappi et al., 1982; Ohsawa et al, 1995; Yu et al., 1998b; Wacnik et al., 2005). Several have focused on the possibility that acupuncture stimulates endogenous opioids (Zhang et al., 1980; Xi et al., 1983; Han et al., 1984).

The first evidence that this is so was provided by Liebeskind's (1973) discovery that electrical stimulation of the PAG caused analgesia of the periphery. Since it was known that morphine-binding receptors were located in the same region of the PAG (Kuhar et al., 1973; Pert and Snyder, 1973), these findings suggested chemical analgesic mechanisms. The subsequent discovery that morphine and other opiates act primarily at the midbrain level of the nervous system (Bausbaum et al., 1977), as well as the existence of enkephalins (Kosterlitz and Hughes, 1975) and other analgesic compounds in the CNS (Hughes, 1975; Hughes et al., 1975) were further breakthroughs.

More types of endogenous opioids were identified soon after: endorphins in the pituitary (Cox et al., 1975; Teschemacher et al., 1975; Li, 1977) and within the cerebrospinal fluid following stimulation of the PAG and acupuncture stimulation (Sjölund et al, 1977) and dynorphin in the hypothalamus and dorsal spinal cord, among other locations (Goldstein et al., 1979; Hökfelt et al., 1984).

All these discoveries supported the hypothesis of an opioid-mediated endogenous pain inhibition. Based on observations of cross tolerance between morphine and electroacupuncture, Han et al. (1980) were the first to suggest both mechanisms of analgesia were mediated by the same receptors.

The role of endogenous opioids in acupuncture analgesia was confirmed using naloxone, a specific opioid receptor antagonist, which partially reversed the analgesic effect of acupuncture on electrical stimulation-induced experimental pain (Mayer et al., 1977) and in patients with chronic pain (Jiang et al., 1978). Naloxone also blocked electroacupuncture-induced inhibition of nociceptive

responses in feline dorsal horn neurons (Pomeranz and Cheng, 1979) and reversed electroacupuncture analgesia in the monkey (Ha et al., 1991). Additional evidence of acupuncture involving an opioid-based mechanism was demonstrated using knockout mice deficient in opiate receptors. These mice exhibited poor electroacupuncture-induced analgesia (Peets and Pomeranz, 1978). Acupuncture analgesia was also potentiated by protection of endogenous opioid peptides using peptidase inhibitors, such as d-amine acids, d-phenylalanine and bacitracin (Ehernpreis et al., 1978; Han et al., 1981; Zhou et al., 1984).

The endogenous opioid receptors are distributed all over the body, but specifically in the peripheral primary afferent terminals and the nociceptive areas of the CNS (Basbaum and Jessell, 2001; Fields et al., 2005). The involvement of the peripheral opioid system in modulating inflammatory pain has been well-documented (Stein, 1991; Stein et al., 2003). Electroacupuncture administered in a rat model of CFA-induced inflammation, local, but not intraperitoneal, injection of naloxone methiodide, a peripherally acting opioid receptor antagonist, eliminated the analgesic effect 30 min after treatment (Sekido et al., 2003). Acupuncture also causes activation of the descending inhibitory pain pathway which activates the PAG to release more β-endorphins and the NRM to release serotonins (Janssens et al., 1988). Intraplantar injection of a β-endorphin antibody and a corticotropin-releasing factor antagonist also reduced electroacupuncture analgesia (Zhang et al., 2005a).

Taken together, these data strongly suggest that inflammatory pain is modulated by peripheral opioids released by electroacupuncture.

Compelling evidence exists that frequency-dependent electroacupuncture analgesia is mediated by the different opioid receptor subtypes (Han, 2003; Kim et al., 2004; Zhang et al., 2004a; Wang et al., 2005). The radioimmunoassay of spinal perfusates following electrical stimulation at low-frequency (2 Hz) demonstrated that electroacupuncture facilitates the release of enkephalin, but not dynorphin, while high frequency (100 Hz) stimulation resulted in the release

of dynorphin, but not enkephalin in the rat (Fei et al., 1987; Han, 2003) and in humans (Han et al., 1991). Under physiological conditions, low-frequency electroacupuncture is mediated by μ- and δ- receptors and high-frequency electroacupuncture by κ-receptors (Han, 2003; Wang et al., 2005). However, in pathological conditions, such as inflammatory (Zhang et al., 2004b) or neuropathic (Kim et al., 2004; Sun et al., 2004) pain, κ-receptors do not appear to mediate electroacupuncture analgesia.

In rats with neuropathic pain, 2 Hz electroacupuncture induced a robust and longer lasting effect than 100 Hz (Kim et al., 2004; Sun et al., 2004). When the arcuate nuclei were lesioned, low-frequency electroacupuncture-induced analgesia, but not high-frequency electroacupuncture, was abolished (Wang et al., 1990). Conversely, selective lesioning of the parabrachial nuclei attenuated high-frequency electroacupuncture-induced analgesia but not low-frequency electroacupuncture (Wang et al., 1991). Thus, different opioid-expressing brain nuclei are involved in electroacupuncture analgesia produced by low- and high-frequency stimulation.

Most of the previously mentioned brain nuclei and regions involved in processing acupuncture signals contain opioid peptides and μ-, δ-, and κ-receptors. At the supraspinal level, selective inhibition of opioid receptors in different areas decreases the analgesic effect of acupuncture (He, 1987).

The PAG is a critical region in the descending pain inhibitory system (Millan, 2002) containing a high density of opioid receptors, specifically μ- or δ-receptors (Xie et al., 1983; Han et al., 1984). Electrical stimulation of the PAG can produce analgesia potent enough to allow for exploratory laparotomy surgery in rats (Reynolds, 1969). Since the axons of the periaqueductal gray neurons project to the NRM, electrical stimulation of the PAG enhances firing of the NRM neurons during the ensuing analgesia (Liu, 1996).

The arcuate nucleus is another important structure in the endogenous opioid peptide system. It is densely populated by beta-endorphin-containing neurons, whose axons project to the PAG and other areas (Takeshige et al.,

1992). Sectioning the β-endophinergic tract or microinjection of naloxone into the PAG blocks excitation of NRM neurons following stimulation of the arcuate nucleus (Yin et al., 1988). This association between the arcuate nucleus, the PAG, and the NRM is one of the reasons why it is implicated in mediating acupuncture analgesia (Wang et al., 1990; Takeshige et al., 1992). Moreover, stimulation of the arcuate nucleus significantly increased electroacupuncture analgesia and electroacupuncture-induced responses of neurons in the dorsal raphe nucleus. Electroacupuncture-induced responses of neurons in the locus coeruleus, on the other hand, were decreased by stimulation of the arcuate nucleus (Yin et al., 1988).

The thalamic anterior nuclei are another portion of the brain densely populated with opioid receptors. They are considered part of the limbic system, and as such involved in pain emotion (Mark et al., 1963; Pert et al., 1976). Electroacupuncture produces naloxone-reversible inhibition of nociceptive responses in neurons of the thalamic anterior nuclei (Dong et al., 1987).

Perhaps one of the most fascinating studies to link the opioid system to acupuncture is the recent work of Harris et al. (2009). These investigators analyzed the short- and long-term effects of traditional Chinese acupuncture versus sham acupuncture treatment on in vivo MOR binding availability in chronic pain patients diagnosed with fibromyalgia. Harris and colleagues utilized PET scans with (11)C - once during the first treatment session and then repeated this again a month later following the eighth treatment. They found that acupuncture therapy evoked both short-term and long-term increases in MOR binding potential, in multiple pain and sensory processing regions including the cingulate (dorsal and subgenual), insula, caudate and amygdala that were absent in the sham control group of patients.

C. Non-opioid modulators in acupuncture analgesia

The first indication of the involvement of neurotransmitters or chemical mediators in acupuncture mediators was that cerebrospinal fluid of donor rabbits receiving acupuncture treatment increased the pain threshold of recipient rabbits (Han et al., 1982). Since this breakthrough finding, numerous human and animal studies have demonstrated that acupuncture analgesia is a complex physiological process mediated by various transmitters and modulators.

Different frequencies of electrical stimulation without moving the position of the needle can affect the release of different neuropeptides. This concept was described by Han et al. (1991), who observed that low frequency (2 Hz) electroacupuncture increases the content of β-endorphin and met-enkephalin in the CSF, while high frequency (100 Hz) accelerated the release of dynorphin.

Numerous non-opioid substances modulate acupuncture and electroacupuncture. Following is a brief description of the most important ones.

Cholecystokinin octapeptide (CCK-8) is widely distributed in the brain and spinal cord, where it produces potent anti-opioid activity via the CCK8 receptor (Ito et al., 1982; Faris et al., 1983; Watkins et al., 1985; Han, 1995; Han, 2003), and has been shown play a role in electroacupuncture analgesia (Chen et al., 1998; Lee et al., 2003; Ko et al., 2006; Huang et al., 2007). Intrathecal administration of CCK-8 and CCK8 receptor antagonists significantly depressed and potentiated electroacupunture-induced antinociception, respectively (Huang et al., 2007). Significantly increased levels of spinal CCK-8 have been quantified in rats experiencing weak analgesic effects (non-responders) following electroacupuncture compared to those with strong electroacupuncture-induced analgesia (responders) (Zhou et al., 1993). Also, high-frequency electroacupuncture increases CCK receptor mRNA in the rat hypothalamus in non-responders (Ko et al., 2006). Individual sensitivity to acupuncture is likely

due to both CCK release and the density of CCK receptors. (Tang et al., 1997; Lee et al., 2002).

Placebo analgesia is mediated by endogenous opioids (Benedetti and Amanzio, 1997), and blockade of CCK receptors, is an important mechanism of placebo analgesia (Benedetti, 1996). Given the modulating effects of CCK on electroacupuncture analgesia, it is possibly that acupuncture analgesia may share a common mechanism with placebo analgesia.

Hydroxytryptamine (5-HT) and its receptor are densely expressed in the CNS, especially the NRM, and play a crucial role in modulating nociception (Millan, 2002; Kayser et al., 2007; Liu et al., 2007). Multiple 5-HT receptor subtypes also exist in the nervous system (Millan, 2002), particularly in the neurons of the dorsal horn in the spinal superficial laminae where primary nociceptive afferent fibers terminate (5-HT1A and 5-HT1B) (Hamon and Bourgoin, 1999), as well as on primary nociceptive afferent fibers (5-HT2A and 5-HT3) (Hamon et al., 1989; Carlton and Coggeshall, 1997). Of these receptor subtypes, 5-HT1A and 5-HT3 play important roles in mediating electroacupuncture analgesia through modulation of substance P release (Hjorth, 1993; Yonehara, 2001; Chang et al., 2004; Kim et al., 2005). The role of 5-HT in mediating acupuncture analgesia has been well-summarized (Han and Terenius, 1982). Electroacupuncture increases the central content of 5-HT and its metabolic products, particularly in the NRM and the spinal cord (Han et al., 1979a, Han et al., 1979b, Ye et al., 1979; Zhu et al., 1997a; Zhu et al., 1997b).

Noradrenalin (NA). It is well-documented that the NA-containing neurons reside in several nuclei of the brain stem involved in pain modulation (Ungerstedet, 1971; Millan, 2002). Electroacupuncture-induced analgesia has been shown alongside decreased NA levels in the rat brain (Dong et al., 1978; Han et al., 1979a; Zhu et al., 1997b). Spinal α2-adrenoceptors play a crucial role in

inhibitory descending pain control by noradrenergic projections from supraspinal nuclei to the dorsal horn (Zhao and Duggan, 1988; Liu and Zhao, 1992; Millan, 2002), particularly in modulating neuropathic pain (Yu et al., 1998a). At the spinal level, injection of the α2 receptor antagonist yohimbine significantly blocked electroacupuncture analgesia in neuropathic rats (Kim et al., 2005). Thus, NA in the brain may have an inhibitory effect on acupuncture analgesia, but not in the spinal cord, where it may actually facilitate acupuncture analgesia.

Glutamate. This excitatory amino acid and its receptors are abundant in nociceptive primary afferent fiber terminals (Li et al., 1997) and play an important role in spinal transmission of nociceptive information and central sensitization (Aanonsen et al., 1990; Dougherty and Willis, 1991; Ren et al., 1992; Song and Zhao, 1993a; Song and Zhao, 1993b; Millan, 1999; Hu and Zhao, 2001; Du et al., 2003).

NMDA receptors are distributed densely in the superficial dorsal horn of the spinal cord where primary nociceptive afferents terminate (Liu et al., 1994; Coggeshall and Carlton, 1998). In a rat model of spinal nerve ligation, electroacupuncture decreased mechanical allodynia (Huang et al., 2004). Following nerve ligation, low-frequency electroacupuncture also reduced the expression of NMDA receptor subtype NR1 in the spinal superficial laminae (Sun et al., 2004). In the CFA-induced inflammation model, electroacupuncture lessened both inflammatory pain and expression of spinal NMDA receptor subtypes NR1 and NR2 (Choi et al., 2005a; Choi et al., 2005b). Low-dose ketamine, a NMDA receptor antagonist, potentiates the anti-allodynic effects of electroacupuncture in a model of neuropathic pain (Huang et al., 2004) and in inflammation model using carrageenan or CFA (Zhang et al., 2005d).

Gamma-Amino-butyric acid (GABA) is an important inhibitory transmitter in the CNS and is involved in multiple physiological and pathological functions. The

GABAa and GABAb receptor subtypes contribute to modulation of pain (Millan, 1999; Millan, 2002), but their role in acupuncture analgesia is still not completely understood and agreed upon (Fan et al., 1982; Cao et al., 1993; Fang et al., 1993).

Systemic administration of a GABAa receptor antagonist reduces acupuncture analgesia (McLennan et al., 1977), whereas intrathecal diazepam binding to GABAa receptors potentiated electroacupuncture analgesia (Pomeranz and Nguyen, 1987). Similarly, microinjection of a GABAa receptor agonist into the PAG significantly suppresses acupuncture analgesia, whereas injection of a GABA synthesis inhibitor potentiates it (Han, 1989). GABA expression in the PAG was increased following electroacupuncture at ST-36 (Fusumada et al., 2007). Acupuncture also raises the pain threshold in rats alongside an increase of GABA concentration in brain (Tang et al., 1988).

Intracerebroventricular administration of GABAb, but not GABAa, receptor antagonists decreases both acupuncture analgesia and GABAb receptor agonist-induced analgesia (Zhu et al., 1990a, Zhu et al., 1990b; Zhu et al., 2002). In addition, GABAa receptor antagonist administration markedly inhibits acupuncture-induced analgesia at the level of spinal dorsal horn neurons, as well as, electroacupuncture-induced depolarization of C-afferent terminals, suggesting involvement of presynaptic inhibition (Li et al., 1993). Taken together, the data suggest that while both GABAa and GABAb receptors are associated with acupuncture analgesia in the spinal cord, only GABAb receptors are involved at the supraspinal level (Zhu et al., 2002).

Substance P is one of the most important signal molecules mediating peripheral (Xu et al., 2000; Zhang et al., 2007b) and spinal nociception (Yaksh et al., 1979; Duggan et al., 1987; Hunt and Mantyh, 2001). Bilateral stimulation of GB-30 using low-intensity electroacupuncture for 30 min depressed the pain response and reduced noxious stimulation-induced elevation of substance P (Zhu et al., 1991), possibly due to the inhibition of its release (Du and He, 1992). Similarly,

tumor-microperfusion experiments of tumor-bearing mice in our laboratory showed that electroacupuncture treatment at ST-36 completely inhibited substance P release in the tumor (unpublished).

Electroacupuncture also decreased substance P release in the trigeminal nucleus caudalis and A-δ afferents in a rabbit model of tooth pulp stimulation (Yonehara et al., 1992). Since opiates are known to inhibit the release of substance P (Yaksh et al., 1980), and acupuncture facilitates the release of endogenous opioids in the spinal cord (Han, 2003), it is conceivable that (acupuncture) stimulation inhibits substance P release by causing the release of opioids, and thus exerts its analgesic effects.

Angiotensin II is a common neuropeptide in the CNS involved in multiple physiological and pathological functions including the modulation of pain (Toma et al., 1997; Han et al., 2000; Tchekalarova et al., 2003). It appears that the effect of angiotensin II on electroacupuncture-induced analgesia is comparable to that of CCK (Shen et al., 1996).

Somatostatin, an endogenous non-opioid neuropeptide, is found in both the periphery and the CNS, where it contributes to modulation of nociception (Sandkuhler et al., 1990; Song et al., 2002). Somatostatin might be involved in electroacupuncture analgesia for neuropathic pain (Zheng et al., 1995; Dong et al., 2005).

Arginine vasopressin. An early study revealed that the hypothalamic paraventricular nucleus plays an important role in acupuncture analgesia (Yang et al., 1992; Yang and Lin, 1992) because of arginine vasopressin (Yang et al., 2006b). Moreover, it appears that arginine vasopressin is responsible for the glutamate-induced enhancement of electroacupuncture analgesia when ST-36 is stimulated (Yang et al., 2006a).

Neurotensin is found in the periaqueductal gray, which is involved in modulation of nociception (Tershner and Helmstetter, 2000; Li et al., 2001). The enhancing effect of neurotensin on electroacupuncture analgesia has been demonstrated using the tail-flick test in rats (Bai et al., 1999). As this analgesic effect was attenuated by naloxone, it is possible that opioid receptors in the PAG may participate in this neurotensin-mediated potentiation of electroacupuncture analgesia.

Dopamine receptor antagonists, such as the antipsychotic drug haloperidol, have been shown repeatedly to potentiate electroacupuncture analgesia (Han et al., 1979b; Xu et al., 1980; Wang et al., 1997; Wu et al., 1990). Receptor binding studies confirmed these findings and suggested that opioid receptors in several brain regions contribute to the potentiating effect of haloperidol on acupuncture analgesia (Wang et al., 1994; Zhu et al., 1995). Activation of dopamine receptors themselves, particularly DA1 receptor, actually may reduce electroacupuncture analgesia (Wang et al., 1999).

Interleukin 1 (IL-1). Peripheral inflammation causes an increase of IL-1 receptor I mRNA expression in the rat periaqueductal gray, which can be reduced by electroacupuncture (Ji et al., 2003). Electroacupuncture also significantly decreases thermal hyperalgesia following attenuated cancer cell inoculation and inhibits the upregulation of IL-1β and its mRNA. Suppression of IL-1β expression, therefore, may explain – at least in part – how electroacupuncture alleviates bone cancer pain (Zhang et al., 2007c).

Acetylcholine. Electroacupuncture causes a simultaneous elevation of the pain threshold and acetylcholine levels in the cortex, caudate nucleus, hypothalamus and brainstem (Guan et al., 1984), as well as enhanced activity of acetylcholinesterase in the spinal cord (Ai et al., 1984). Acupuncture also has

been shown to significantly enhance the activity of acetylcholine esterase in the jejunum resulting in increased gastrointestinal myoelectrical activity (Niu et al., 2007).

D. Cellular and genetic mechanisms of acupuncture

Spinal cord glia (microglia and astrocytes) are involved in the development and maintenance of inflammatory and neuropathic pain (Song and Zhao, 2001; Ma and Zhao, 2002; Deleo et al., 2004; Ledeboer et al., 2005; Watkins et al., 2005; Zhang et al., 2005c; Sun et al., 2007a; Watkins et al., 2007; Zhang et al., 2007c). Electroacupuncture and disruption of glial function synergistically suppress inflammatory pain in arthritic rats (Sun et al., 2006; Sun et al., 2007b). Acupuncture also attenuates the increase in MAC-1, a marker of microglial activation, and reduces the expression of COX-2 and inducible nitric oxide synthase (iNOS) in a model of Parkinson's disease (Kang et al., 2007). Recent work also shows that acupuncture enhances the differentiation of bone marrow derived stromal cells into astrocytes and neurons in the treatment of spinal cord injury (Sun et al., 2009) and upregulates insulin-like growth factor-I (IGF-I) expression in dorsal root ganglia and in the spinal cord dorsal horn (Dai et al, 2009) and neurotrophin 4 expression in the DRGs (Liu et al., 2009). Since IGG-1 and NT-4 are found in glial cells, acupuncture appears to upregulate the expression of several key proteins in glia, which may contribute to its antinociceptive effect as well as to its effects on other spinal cord mechanisms.

G protein-coupled receptors, such as opioid receptors mediate anti-nociception, and, thus, are a focus of attention in pain management (Przewlocki et al., 1987). Following the destruction of G protein function and signal transduction using pertussis toxin, electroacupuncture was ineffective in producing anti-hyperalgesic effects in a rat model of CFA-induced inflammatory pain (Liu et al., 2005).

Extracellular signal-regulated protein kinase (ERK) mediates intracellular signal transduction involved in cell proliferation, differentiation, neuronal plasticity, and nociception (Ji et al., 2002; Obata and Noguchi, 2004). Electroacupuncture at contralateral ST-36 may inhibit ERK-positive neurons in the spinal dorsal horn (Song et al., 2006).

Gene expression and genetic factors. Electroacupuncture markedly induces a rapid expression of the c-fos gene in the spinal cord and various brain regions, suggesting that transcription factors are also involved in processing acupuncture signals (Ji et al., 1993a; Ji et al., 1993b; Guo et al., 1996).

Based on the cDNA microarray analysis data collected in the rat neuropathic pain model, multiple signaling pathways, including opioid receptor- and MAP kinase-mediated pathways, as well as several other genes, are involved in pain development and electroacupuncture analgesia (Ko et al., 2002).

Inherited genetic factors may also explain individual differences in acupuncture response and electroacupuncture frequency-dependency in acupuncture analgesia (Mogil, 1999; Chae et al., 2006). A genotype sensitivity to electroacupuncture analgesia has been demonstrated in 10 common inbred mouse strains, including Balb-c mice (Wan et al., 2001).

IV. Linking (Electro-) Acupuncture to (Bone) Cancer

A recent evidenced based review concluded that “A great body of data emerging from scientifically sound clinical trials prove that defined complementary procedures are beneficial for oncology patients.” (Beuth and Schlerholz, 2007). However, it is important to differentiate between "alternative" therapies, often promoted falsely as viable options to mainstream lung cancer treatment, and

complementary therapies, adjunctive, effective techniques that treat symptoms associated with cancer and its mainstream treatment. In this regard, both Cassileth et al. (2007) and Lu et al. (2008) concluded in recent reviews that complementary therapies, including acupuncture, play an increasingly important role in the control of symptoms, such as pain, associated with cancer and cancer treatment. Thus, from a clinical standpoint, needling acupuncture and electroacupuncture are advocated in both human and veterinary medicine as adjunct therapies to treat the adverse effects of cancer treatment, or to contribute to multimodal pain management (Staud and Price, 2006). However, neither technique has been investigated adequately as sole therapy for cancer pain.

Electroacupuncture applied to the ST-36 acupuncture point produces analgesic effects in a rat model of mechanical hyperalgesia (Lee and Beitz, 1992) and in a mouse model of chemical hyperalgesia (Chang et al. 2004). The same acupuncture point is popular for its anti-inflammatory effects as demonstrated by Kim et al. (2006) in their murine model of air pouch inflammation. In addition, electroacupuncture at ST-36 affects interferon-γ levels and subsequently NK cell activity, which suggests immune-boosting effects (Yu et al., 1998b). Recently, inflammation was linked to tumerogenesis when selective cyclooxygenase-2 (COX-2)-inhibiting nonsteroidal anti-inflammatory drugs, such as meloxicam, exhibited antineoplastic effects in vitro (Naruse et al., 2006; Wolfesberger et al., 2006). Lin and Karin (2007) extensively reviewed this extraordinary finding. In effect, the inducible enzyme COX-2 is overexpressed in canine (Mullins et al., 2004) and human (Masi et al., 2007) osteosarcoma cells, and electroacupuncture significantly decreases COX-2 expression in different models of pain and hypersensitivity (Lau et al., 2008) and inflammation (Lee et al., 2005; Lee et al., 2006).

Recent studies have shown the importance of ERK in osteosarcoma cell differentiation and metabolism (Alkhalaf and Jaffal, 2006; Shimo et al., 2007; Tang et al., 2008). Given that electroacupuncture is able to inhibit ERK

expression (Song et al., 2006), this may represent another pathway by which electroacupuncture may interfere with osteosarcoma growth and function.

In addition, in the periphery, deep acupuncture stimulation at ST-36 induces a decrease in sympathetic renal nerve activity and mean arterial blood pressure in anesthetized rats (Ohsawa et al., 1995). Electroacupuncture applied at the ST-36 acupuncture point also significantly raises the plasma levels of β-endorphin, β-lipotropin and ACTH (Nappi et al., 1982). Therefore, it is conceivable that any changes in circulation may affect tumor growth.

Moreover, in hyperalgesic mice, tumors tend to exhibit increased CGRP-immunoreactive nerve fibers and reduced vascularization (Wacnik et al., 2005). Using a pain model of tetanized gastrocnemius muscle in guinea pigs, Takeshige and Sato (1996) found that needling of the muscle stimulated a variety of sensory nerve endings, including those containing CGRP. Twice-weekly treatments with acupuncture reduce the number of CGRP-immunoreactive nerve fibers in the skin being needled, indicating that the pain-relieving effects of acupuncture depend in part on peripheral innervation (Carlsson et al., 2006). Thus, it is quite possible that electroacupuncture has the ability to affect local vascularization and innervation associated with tumor growth.

Huang et al. (2002) described the use of electroacupuncture at the ST-36 acupuncture point in mice in detail. This particular acupuncture point exhibits analgesic, anti-inflammatory, and immune-boosting effects in several species (Lee and Beitz, 1992; Yu et al., 1998b; Chang et al., 2004; Kim et al., 2006). In consideration of the recent interest in inflammation and its role in tumerogenesis (Naruse et al., 2006; Lin and Karin, 2007), electroacupuncture could potentially alter osteosarcoma growth and metastasis through its anti-inflammatory effects. In this regard, a recent study by Lai et al. (2008) suggests that acupuncture applied at ST-36 can increase immune function and inhibit the tumor growth of Walker-256 tumor cells in vivo. If this is true, then electroacupuncture could provide a novel alternative treatment option to traditional chemotherapeutic approaches.

PART II. EXPERIMENTAL WORK

5. METHODOLOGY & RESULTS

I. GENERAL METHODOLOGY

In the following studies, mice were implanted with K7M2 osteosarcoma cells and tested for primary hyperalgesia using von Frey filaments applied to the plantar surface of the paw. Tumor size was measured using calipers at selected timepoints following implantation. Electro-acupuncture treatment was applied to the ST-36 acupuncture point using different intensities at different time intervals to determine if electroacupuncture regimes differentially, but simultaneously, affect tumor growth, metastasis and/or tumor-induced nociception in an intensity-dependent fashion. Following transcardiac perfusion with fixatives, tumor tissue was harvested and processed for histopathology or immunocytochemistry. Spinal cords were collected and processed for c-fos immunohistochemistry. In addition, this tumor model was used to evaluate if there are gender differences in tumor-induced nociception and in electroacupuncture-induced anti-nociception.

Approval for the research project was obtained from the Institutional Animal Care and Use Committee (IACUC) of the University of Minnesota.

A. Tumor Implantation

Animals. The Balb-cAnNcR (Balb-c) mouse strain is susceptible to tumor development, particularly osteosarcomas and was, therefore, selected to perform the proposed studies (Khanna et al., 2000). Furthermore, the tumor cells used were passaged originally in Balb-c mice and would recognize this mouse strain as a natural host.

The number of animals used for all projects outlined above was determined by the need to obtain a large enough sample size to ensure uniform

sampling and achieve statistical significance. The general criteria for determining sample size for the present animal experiments were based on sample size calculations for parametric dependent measures and utilize standard deviations derived from previous experiments in our lab, as well as from those reported in the literature. The number of animals was determined based on sample size calculations to generate statistical power for the detection of significant differences. Actual sample size for experimental and control groups varied according to experimental design, but were generally 10 mice for behavioral, and 6-8 mice for immunohistochemical and histopathological studies. This was consistent with the previous literature (Wacnik et al., 2001).

Young adult male and female Balb-c mice (4-5 weeks old) were used in the following studies. All mice were obtained from a reputable breeder (NCI) to ensure a uniformly homogenous and consistent response to treatment. Following arrival at the Research Animal Resources (RAR) housing facilities of the University of Minnesota, the mice were left undisturbed for 3 days to acclimate to their new surroundings. Animal care and management was provided by RAR.

Tumor Model. The murine hindpaw model using fibrosarcoma cells was developed in the Beitz and Wilcox laboratories and has been employed repeatedly for a variety of cancer and pain studies (Wacnik et al., 2000; Wacnik et al., 2001). No one has used this model in association with electroacupuncture and osteosarcoma, but the calcaneus bone served as an ideal site for implantation of the aggressively invasive K7M2 osteosarcoma tumor cells.

The hindpaw model has been validated and is a cost- and time-effective way to study cancer biology. Intraosseous cell implantation ensured more significant bone involvement and placement of the cells exactly where they were desired. This model had the advantage over femoral or humeral implants, in that tumors could be verified visually as early as 7 days post implantation and tumor growth could be followed using external measurements. Furthermore, the location of the tumor in the calcaneus was particularly convenient, as it provided

ready access for the measurement of primary hyperalgesia, which is more difficult to measure in bone tumors involving the femur or tibia.

Tumor Cells. The tumor cells used were host-specific K7M2 osteosarcoma cells, a gracious donation by Dr. Chand Khanna of NCI. The cells were chosen for their increased aggressive and invasive character compared to the slower growing K12 osteosarcoma cell line (Khanna et al., 2000). This characteristic was particularly attractive, as it allowed for a reduced study period of only a few weeks versus several weeks to months (Khanna et al., 2000).

Tumor cells were grown in Dulbecco's Modification of Eagles Medium (DMEM[11]) fortified with 10% Fetal Bovine Serum[12] (in 5% carbon dioxide at 37^{o}C) and maintained according to standard cell culture techniques.

Tumor Cell Implantation. Mice were anesthetized in a plexiglass anesthetic chamber using 3% isoflurane in 3L/min oxygen (Figure 3). Once each animal was motionless and breathing had steadied, it was removed from the box and fitted with a face mask delivering 2% isoflurane in 2 L/min oxygen. Surgical plane of anesthesia was determined when the animal demonstrated non-responsiveness to paw pinch. Mice were then implanted with 2 x 10^5 osteosarcoma cells (controls: saline-vehicle) using a 29 gauge, sterile single use needle attached to a 0.3 mL insulin syringe to bore manually through the left calcaneus bone. Cells were injected in a volume of 10 µL as previously described (Wacnik et al., 2001). Mice showing signs of motor dysfunction at any point after the implantation of tumor cells were euthanized and not included in the study.

[11] GIBCO®, Invitrogen
[12] ATTC, Manassas, VA

B. Electroacupuncture Treatments

One of the advantages of electroacupuncture over traditional manual acupuncture is that it can produce a stronger stimulation without causing tissue damage associated with twirling and lifting and thrusting the needle (Mayor, 2007). In addition, it is easier to control the frequency of the stimulus and the amount of stimulus compared with hand manipulation of the needles.

The use of electroacupuncture at ST-36 was described for mice in detail by Huang et al. (2002). Electroacupuncture was performed on mice anesthetized with isoflurane using a pair of stainless steel needles inserted 3 mm deep into the ST-36 acupuncture point and connected to an electric pulse generator. Stimulation parameters for the electroacupuncture treatment were set at a low frequency of 4 Hz (7 V, 5 ms duration pulses, 1 ms pause), since low frequencies repeatedly were found to be more effective than high (100 Hz) frequencies (Lee and Beitz, 1993; Huang et al., 2002). Stimulation was delivered for 30 minutes (Mao-Ying et al., 2006).

Sham electroacupuncture consisted of acupuncture needles being inserted into the ST-36 for 30 minutes, but without application of electrical stimulation. We decided to not include a sham group with non-penetrating sham acupuncture needles or a group with non-acupoint stimulation, since a previous study showed no significant differences in brain activation when shallow and deep needling were compared to sham needles using fMRI (Chiu et al., 2003). This fMRI study looked at LI-4, ST-36 and a number of other acupuncture points. With one exception, ST-36 activated the same brain structures as LI-4 (Chiu et al., 2003).

In addition, the Nordic Cochrane Center recently conducted a substantial, systematic review of 13 three-group acupuncture studies covering a variety of painful conditions in humans (Busko, 2009). Using a 100-mm visual analog scale to record pain, true acupuncture reduced the pain score by only 4 mm more than sham acupuncture did, indicating that manual acupuncture has minimal analgesic effect over sham acupuncture.

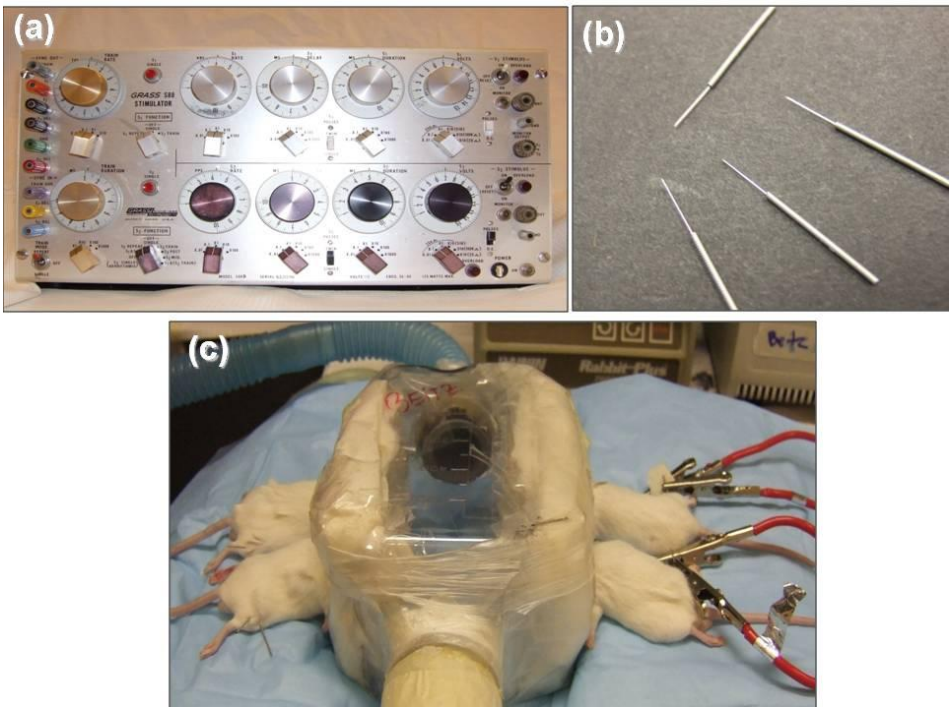

Figure 1. Electroacupuncture (EA) equipment and setup. (a) Grass S88 stimulator; (b) Acupuncture needles; (c) Balb-c mice under isoflurane anesthesia in a custom-made anesthetic chamber accommodating 4 mice simultaneously. Sham treated mice are on the left and EA mice on the right.

Furthermore, German researchers conducted a direct comparison of true acupuncture and sham acupuncture at a non-acupoint, as well as conventional therapy, in patients with chronic low back pain (Haake et al., 2007). After 6 months of bi-weekly 30-minute sessions, the response rates to true and sham acupuncture were 47.6% and 44.2%, respectively. Either acupuncture treatment was almost twice as effective as the conventional therapy (27.4%).

Furthermore, when the efficacy of acupuncture in fibromyalgia syndrome (FMS) was reviewed, the conclusion was that acupuncture and sham acupuncture (superficial needling or needling away from acupuncture points) had similar effects (Lundeberg and Lund, 2007). This could be explained by the expanded receptive fields of central neurons due to central sensitization resulting in a larger topographic distribution of the pain. The same also occurs following

repeated nociceptive input from needled muscles. Consequently, the effects of sham acupuncture at a non-acupoint may mirror those produced by needling a specific acupuncture point. This implies that superficial needling or needling away from specific acupuncture points is not inert.

Finally, since acupuncture analgesia is dependent on de-Qi (Wang et al., 1985; Pomeranz, 1989; Haker and Lundeberg, 1990; Hui et al., 2005) and the mechanical signal elicited by manipulation of the needle (Gunn, 1978; Langevin et al., 2001; Langevin et al., 2002), mere placement of a needle into an acupuncture point should result in minimal to no stimulation.

Therefore, what will be termed "sham treatment" in the following experiments actually consisted of placement of an acupuncture needle at ST-36, but without electrical stimulation or manual needle manipulation.

The electroacupuncture treatment groups used in this research project are outlined in Table 1.

Transcardiac perfusion. Each mouse was anesthetized deeply with sodium pentobarbital[13] (100 mg/kg) injected intraperitoneally. When the mouse no longer responded to paw pinch, it was placed on its back in a dissection tray, and the thoracic cavity was quickly accessed via the abdomen to isolate the heart. A 21 G butterfly catheter[14] was inserted into the left ventricle and secured using an alligator clamp. The right auricle or atrium was punctured to allow for drainage of blood and fixative, and the mouse was perfused using 15 mL of Phosphate Buffered Saline (PBS) followed by 30 mL 4% paraformaldehyde[15] in PBS at a rate of 3 mL/min. Following perfusion, tumors, lungs, and spinal columns were excised and post-fixed in the same fixative overnight at 4^{o}C. The next day, samples were cryoprotected in 30% sucrose in PBS for 24-48 hours at 4^{o}C (Standard Transcardiac Perfusion Protocol utilized in the Beitz Laboratory).

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[13] Nembutal®, OVATION Pharmaceuticals, Inc., Deerfield, IL
[14] Terumo® Medical Corporation, Somerset, NJ
[15] Electron Microscope Sciences, Fort Washington, PA

Study endpoints. Tumor-bearing animals were perfusion-fixed with paraformaldehyde under deep anesthesia or euthanized at various timepoints for up to 21 days post-tumor cell implantation. No animals implanted with osteosarcoma cells were kept beyond the 21-day post-implantation period to avoid prolonged or increased pain that might have arisen due to excessive tumor growth. If osteosarcoma tumors grew to a size greater than 1 cm in diameter or if the animals exhibited high levels of pain or distress prior to the 21-day time point, they were euthanized according to RAR and IACUC recommendations.

Statistics. Complete statistical analyses of all data sets were carried out. Comparisons between groups were carried out using a Repeated Measures ANOVA. For single time-point comparisons between groups or within a group, an unpaired Student-t test was employed. Where necessary, paired Student-t test was used to test for statistical significance of individual time points. Post-hoc analyses were carried out when p value was borderline. Data was analyzed and graphed using Microsoft Excel® software. The level of significance was set at $p \leq 0.05$.

GROUP	Designation	TREATMENT
1. Vehicle, no EA	SAL∅	Negative control
2. Osteosarcoma, no EA	OSA∅	Positive control
3. Osteosarcoma + EA	OSA+1	Once, day 1 post implantation
4. Osteosarcoma + sham (same point, no current)	OSA-1	Once, day 1 post implantation
5. Osteosarcoma + EA	OSA++	Days 3, 7, 10, 14, 17 and 21 post implantation
6. Osteosarcoma + sham (same point, no current)	OSA--	Days 3, 7, 10, 14, 17 and 21 post implantation
7. Osteosarcoma + EA	OSA5+	Days 5, 8, 12, 15, and 19 post implantation
8. Osteosarcoma +sham (same point, no current)	OSA5+	Days 5, 8, 12, 15, and 19 post implantation
7. Osteosarcoma + EA	OSA+	Days 7, 14 and 21 post implantation
8. Osteosarcoma + sham (same point, no current)	OSA-	Days 7, 14 and 21 post implantation
9. Osteosarcoma + EA	OSA7+	Days 7, 10, 14, 17 and 21 post implantation
10. Osteosarcoma + sham (same point, no current)	OSA7-	Days 7, 10, 14, 17 and 21 post implantation
11. Osteosarcoma + EA	PxEA+	3 EA treatments every other day prior to tumor implantation
12. Osteosarcoma + sham (same point, no current)	PxEA-	3 sham treatments every other day prior to tumor implantation

Table 1. Overview of the treatment groups, EA/sham treatments, and numbers of animals expected to be used. EA = electroacupuncture; OSA = osteosarcoma

II. SPECIFIC METHODOLOGY

1. Does electroacupuncture affect tumor growth in mice with experimentally-induced osteosarcoma?

a) Does electroacupuncture have a tumor-enhancing or tumor-inhibiting effect and is this dependent on specific electroacupuncture treatment strategies?

A. Tumor Measurement

Ankle width was measured using calipers every other day following implantation. A baseline measure was established prior to tumor injections, and tumor size was continually recorded throughout the duration of the study.

B. Tumor Size Results

Implantation K7M2 osteosarcoma cells into the calcaneus bone of the hind paw in mice, resulted in an average tumor take of 81.46% (86.52% in females and 76.40% in males). It allowed for and detection of tumor growth as early as 7 days post implantation.

The primary tumor in the hind paw was grossly visible, usually as a rounded mass occupying the heel of the foot. Frequently, the tumor was not well defined, but had spread to involve the entire foot (Figure 2 b) and sometimes even dorsally along the tibia.

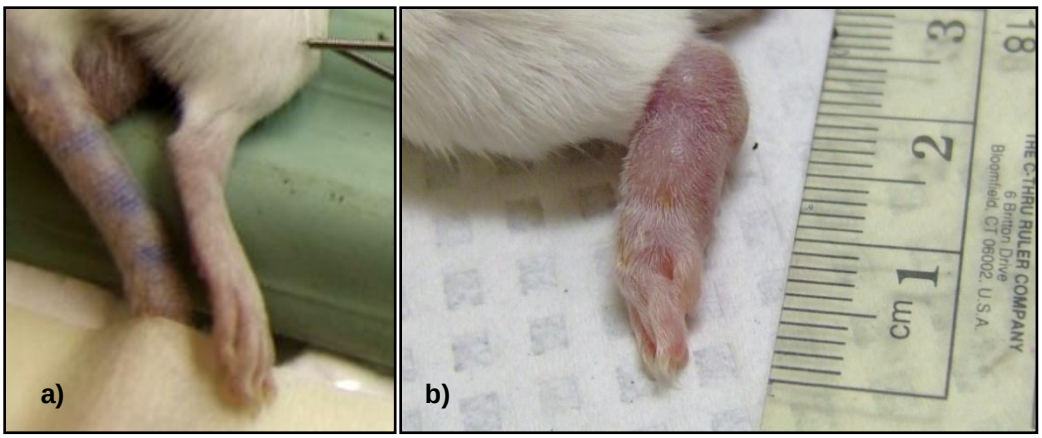

Figure 2. a) Normal sized ankle in adult Balb-c mouse. **b)** Typical appearance of tumor growth in a tumor control (OSA∅) mouse 21 days post implantation. In addition to the heel, the tumor has spread along the length of the paw towards the toes.

Tumor growth was comparable between males and females. With the exception of the tumor controls (OSA∅) and animals receiving electroacupuncture or sham treatments starting on Day 14 (OSA7+ and OSA7-, respectively), or prophylactic treatments prior to implantation (PxOSA+ and PxOSA-), no significant differences were observed between the two sexes (Table 2). In 5 more instances (OSA-1, OSA5+, OSA5-, OSA+, OSA-), greater tumor growth appeared to occur in females, but no statistical significance could be shown. In both males and females, the OSA-1 treatment group was the only one not significantly different from tumor controls (Figure 3 a).

Male mice receiving electroacupuncture treatment administered on Day 1 following implantation (OSA+1), exhibited tumor growth comparable to the tumor controls. Females in the same treatment group experienced a significantly lower percentage growth than OSA∅ animals. With the exception of OSA++, tumor growth appeared to increase between Day 10 and Day 12 in all implanted animals, regardless of treatment (Figure 3 b).

Tumor growth was significantly reduced in all mice receiving electroacupuncture treatment twice weekly starting on Day 3 or Day 5 (OSA++ and OSA5+), or those that received prophylactic treatments (PxOSA+ and PxOSA-). Regular needling in OSA-- mice did not inhibit the tumor, at all. In fact, tumors in this group were larger than tumor controls. The most striking tumor growth – more than 100% for most – occurred in mice receiving once weekly acupuncture treatments starting on Day 7 (OSA+) or Day 14 post implantation.

This indicates that early (and frequent) treatment interferes with tumor growth.

	Increase in ankle width (%)	
Treatment	**Male**	**Female**
SAL∅	1.04	0.61
OSA∅	29.24	48.66
OSA+1	38.23	25.63
OSA-1	46.87	56.69
OSA++	11.31	8.71
OSA--	79.98	65.67
OSA5+	13.04	15.57
OSA5-	15.18	22.96
OSA+	98.76	104.37
OSA-	62.29	64.38
OSA7+	124.44	141.94
OSA7-	143.88	102.93
PxOSA+	14.18	23.17
PxOSA-	14.10	22.69

Table 2. Percentage increase in ankle width from Day 0 to Day 21. Tumor growth was comparable between males and females, with the majority of treatment groups being significantly different (greater or smaller) from the control group. Paired and Unpaired Student-t test. $p \leq 0.05$.

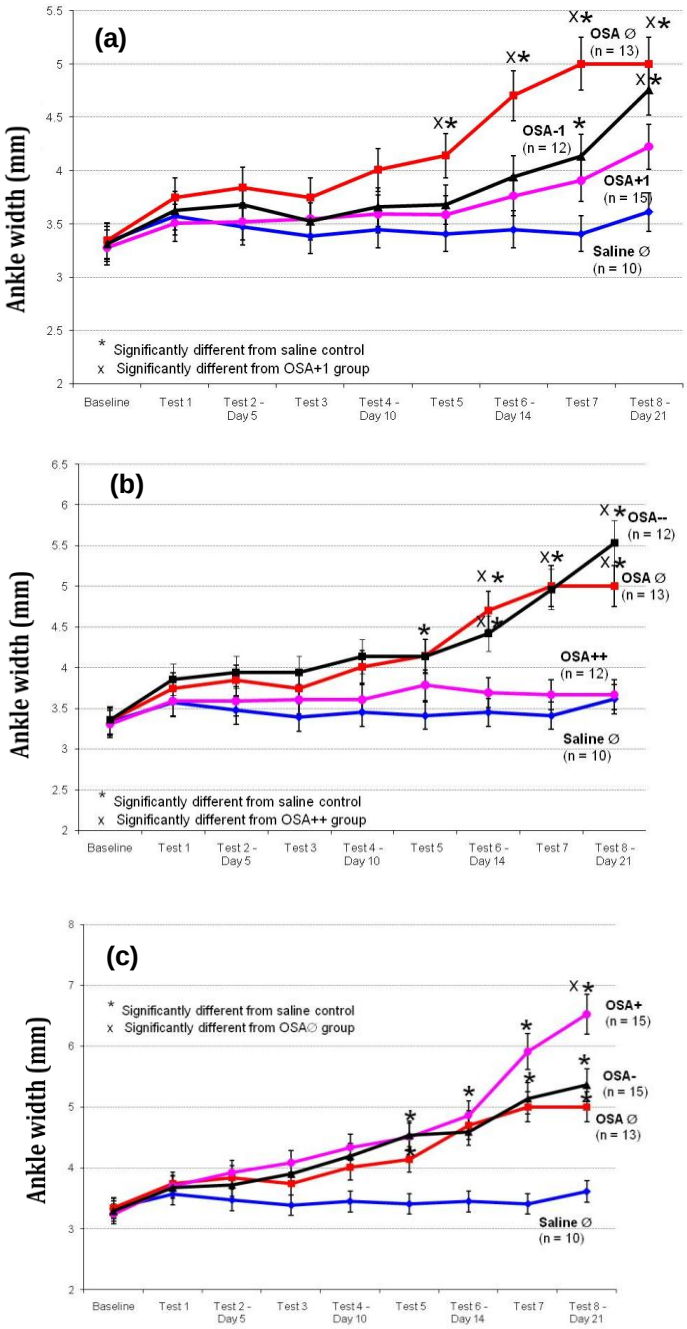

Figure 3. Tumor growth over the 21-day study period. Pooled data from both male and female mice. (a) OSA+1 and OSA-1, single treatment 1 day post implantation. (b) OSA++ and OSA--, twice weekly treatments for 21 days starting on Day 3 post implantation. (c) OSA+ and OSA-, once weekly treatments for 21 days starting on Day 7 post implantation. ANOVA for repeated measures with post-hoc comparison employing Bonferroni test. Unpaired Student-t test. $p \leq 0.05$ with 5% error.

b) Does electroacupuncture cause changes in the innervation and vascularization of osteosarcoma tumors in a way that could impact tumor growth?

A. Tumor Staining

A separate set of mice underwent all treatments as laid out in Table 1 above, but transcardiac perfusions were performed using Zamboni's fixative (2% paraformaldehyde, 15% saturated picric acid, 25% PBS, 50% phosphate buffer, distilled water to bring volume to 100%, 2N sodium hydroxide to alkalinize). Tumors were harvested and post-fixed in Zamboni's.

In collaboration with Dr. Marna Ericson's laboratory in the Department of Dermatology, tumors were sectioned and immuno-stained with specific antibodies to nerves and vasculature (Ericson et al., 1999; Wacnik et al., 2005). Following is the multi-staining protocol for laser scanning confocal microscopy, as provided by Dr. Marna Ericson. Tumor tissue fixed with Zamboni's was mounted in OCT (optimal cryostat temperature, Electron Microscopy Sciences) and cut on a cryostat into sections 150 microns thick. Cut tumor sections were kept in PBS to avoid tissue drying and then washed with TX/PBS (0.3 % Triton-X100 in PBS). The tissue was incubated in 5% normal serum in TPBS[16] (5%-NTX/PBS) overnight at room temperature under continuous gentle gyro-rotatory shaking.

The next day, the 5% NTX/PBS was discarded. Fifty to 100 microliters of primary antibody (PGP-Rb for tumor nerve innervation, CD31-Rt for blood vessels, and LYVE1-Gt for lymphatics) in 1% NTPBS was added at a pre-determined dilution and the tissue was incubated overnight at room temperature with gentle gyro-rotatory shaking.

[16] Jackson Immunoresearch, Inc.

On the third day, tissue sections were washed 3-4 times with 1% NTPBS over an 8-hour period. Afterwards, the 1% NTX/PBS was discarded and all sections were incubated in 100 microliters of secondary antibody (DaRb**CY5** for tumor nerve innervation, DaRt**CY2** for blood vessels, and DaGt**CY3** for lymphatics) at the pre-determined dilution overnight (and protected from light) at room temperature with gentle gyro-rotatory shaking.

On Day 4, tumor sections were washed 3-4 times with 1% TX/PBS and incubated overnight at room temperature with gentle gyro-rotatory shaking.

The next day, tissues were treated with nuclear stain (DAPI[17]) for 5-10 minutes, then washed in PBS and incubate at room temperature with gentle gyro-rotatory shaking for one hour. The tumor sections then were examined under the dissection microscope and any debris was removed. Using the dissecting microscope, the tissue was placed onto coverslips and carefully submerged in 1.35% - 1.5% Noble Agar[18] in distilled water. After the agar had solidified, tissue-mounted coverslips were dehydrated for at least 30 minutes each, first, in 75% ethanol, followed by 95% ethanol, and finally, in 100%, ethanol. To enhance the optical clarity of the specimen and reduce the refractive index differences between sample, coverslip and microscope objective, coverslips were immersed in methyl calculate[19] for at least 30 minutes. Finally, the coverslips were mounted onto slides using DPX or DEPEX mounting medium,[20] and dried protected from light for 48 hours.

Prepared samples were scanned using the Olympus FLUOVIEW 1000 Upright confocal microscope available at the Biomedical Image Processing Lab (BIPL) of the University of Minnesota. The added benefit of confocal microscopy is that it makes scanning tissue in 3-D – a series of tissue layers superimposed on top of each other – possible.[21] This allows for a more realistic quantification,

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[17] 4',6-diamidino-2-phenylindole
[18] Sigma-Aldrich®, Inc.
[19] Sigma Co. Inc.
[20] Electron Microscope Sciences, Fort Washington, PA
[21] Olympus FLUOVIEW 1000 Confocal Microscope Manual, available at http://bipl.umn.edu/

as most anatomical structures (such as nerves and blood vessels) traverse the tissue at different planes.

For quantification, tissue was scanned at a higher magnification (20x) to allow for better resolution of nerve fiber and blood vessel or lymphatic branching. Each specimen was scanned twice at 2 different sites (Figure 4) and the results averaged for a more uniform distribution. Confocal scan files were extracted to *.TIFF format using ImageJ[22]. Each TIFF image was opened individually in Adobe Photoshop CS3 to outline the relevant areas to be counted, which were then exported as individual layers for each structure (nerve, blood or lymph vessel). The actual count was carried out using ImageJ once more.

Quantitative analysis[23] was carried out using the Adobe Photoshop Extended CS3 version 10.0 and ImageJ programs, followed by ANOVA for repeated measures.

B. Innervation and Vascularization Results

Tumor sections scanned with Laser Scanning Confocal Microscopy showed clear anatomical structures at 4x magnification (Figures 4 and 5).

Animals in treatment groups characterized by increased tumor growth or size (such as OSA+) had more defined tumors or more extensive destruction of normal tissue. In severe cases, where the tumor approached 10 mm in diameter, virtually no subcutaneous tissue was identifiable (OSA+, Figure 5 a) at low magnification. Conversely, early and frequent electroacupuncture (OSA++) preserved the anatomical structures and contained the tumor compared to tumor control and OSA+ treatment groups (Figure 5 a, c, d).

[22] Available through the BIPL website: http://bipl.umn.edu/downloads
[23] Analysis macros were custom designed by BIPL staff.

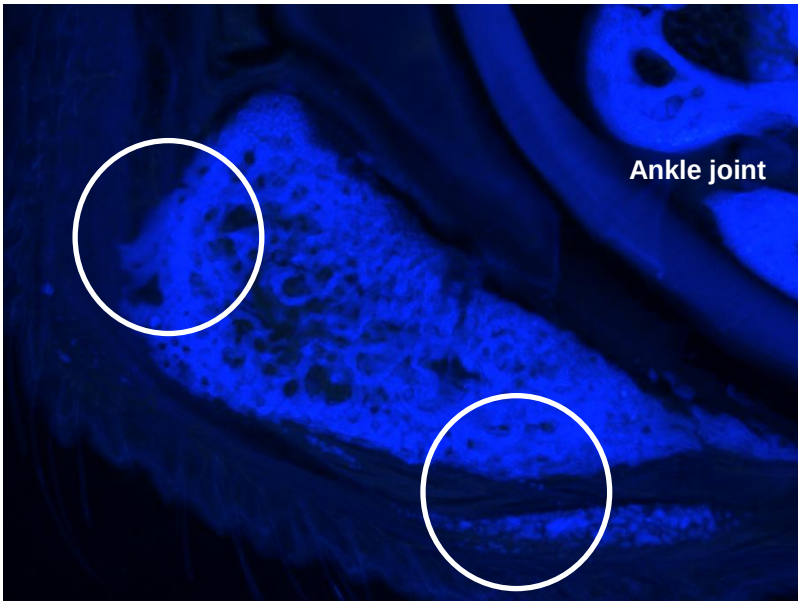

Figure 4. Laser Scanning Confocal Microscopy photomicrograph of 150 micron thick section through the left rear heel (4x). For each specimen, magnified (20x) scans were taken at either end of the calcaneous/tumor as indicated by the encircled areas. Fiber density was measured on both the tumor and skin sides, and averaged. DAPI nuclear stain.

Innervation. Early and frequent electroacupuncture and non-stimulated needling (OSA++, OSA--, OSA5+) significantly decreased both subcutaneous and tumor innervation (Figure 6 a). Interestingly, OSA- also caused a significant decrease. Apparent decreases in tumor innervation, albeit not statistically significant, were also observed in OSA5-, OSA7+, and both prophylactic treatment groups.

This would indicate that the frequency of treatment may be a determining factor, in addition to early interference with tumor growth or establishment.

Vascularization and Lymphatics. Subcutaneous and tumor vasculature did not change significantly between treatments, except for the OSA++ group, which had

significantly less vascular structures when compared to the tumor control (Figure 6 c). Interestingly, OSA-- also caused a significant decrease in subcutaneous vasculature, indicating that mere needle placement early on, even without stimulation, may exert some effect. The most abundant vessels were the subcutaneous lymphatics, which remained – with few exceptions – comparable to controls throughout the study. Both OSA++ and OSA+, as well as OSA7- and the two prophylactic treatments, did have fewer subcutaneous lymph vessels (Figure 6 b). With few exceptions, tumor lymphatics remained unchanged.

This implies that the osteosarcoma is able to sustain a steady network of blood and lymphatic vessels around its periphery in spite of electroacupuncture treatment. However, early and frequent electroacupuncture has the ability to reduce both subcutaneous and tumor innervation and lymphatics associated with osteosarcoma. While the effects of acupuncture on the number of cutaneous CGRP-immunoreactive nerve fibers has been documented previously (Carlsson et al., 2006), this is the first documentation of the effects of electroacupuncture on lymphatics.

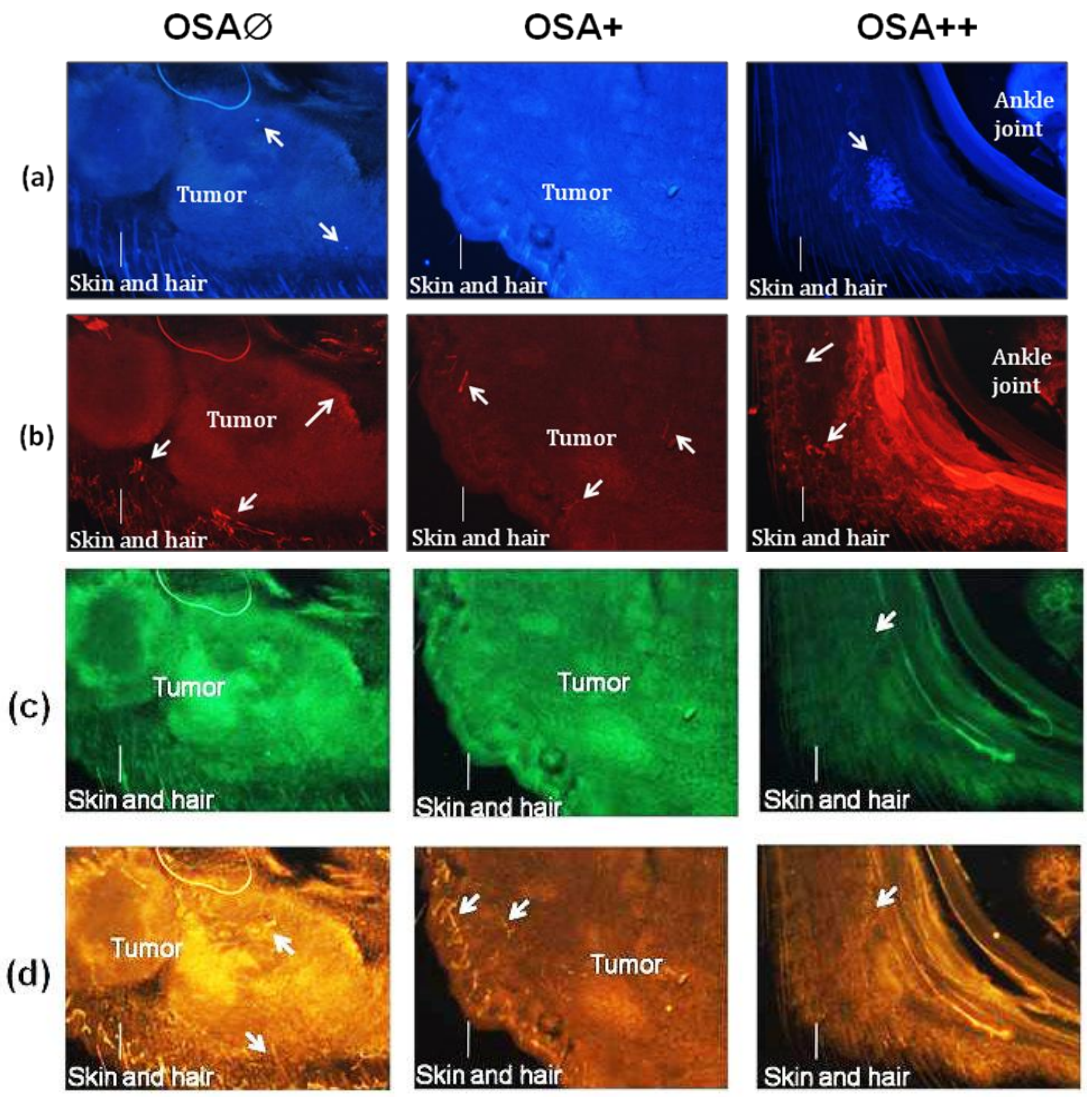

Figure 5. Representative photomicrographs of 150 micron thick sections through the left rear heel of mice from groups with the greatest significant differences in hypersensitivity and tumor growth/size. Laser Scanning Confocal Microscopy (4x). (a) DAPI nuclear stain; (b) Tumor nerve innervation: PGP-Rb/DaRb**CY5** stain; (c) Vasculature: CD31-Rt/DaRt**CY2** stain; (d) Lymphatics: LYVE1-Gt/DaGt**CY3** stain. Arrows indicate presence of positively stained structures.

c) Does electroacupuncture alter tumor metastasis to the lungs?

A. Staining of Lungs

Following transcardiac perfusion, both lungs were harvested for localization of metastasis. After fixation, lungs were refrigerated in 30% sucrose in PBS until examination. Lungs were injected via the trachea with India Ink solution (15% India Ink, 85% water, 3 drops NH4OH/100 ml) and washed in Feket's solution (300 ml 70% EtOH, 30 ml 37% formaldehyde, 5 ml glacial acetic acid) and incubated in fresh Feket's overnight at 4^{o}C.

The following day, the lungs were washed repeatedly (3-5) times in fresh Feket's solution before being examined for presence of white metastatic nodules and areas of lung pathology or consolidation under the dissection microscope (Anderson et al., 1990; Khanna et al., 2000).

B. Results

All of the harvested lungs were examined grossly first. Those which exhibited distinct areas of white or grey discoloration involving parts of or complete lung lobes were referred to the histopathology laboratory for histological examination. However, histopathological findings were inconclusive. The results suggest that at the time point we selected for harvesting the lungs (Day 21 post-implantation), no detectable metastasis had occurred.

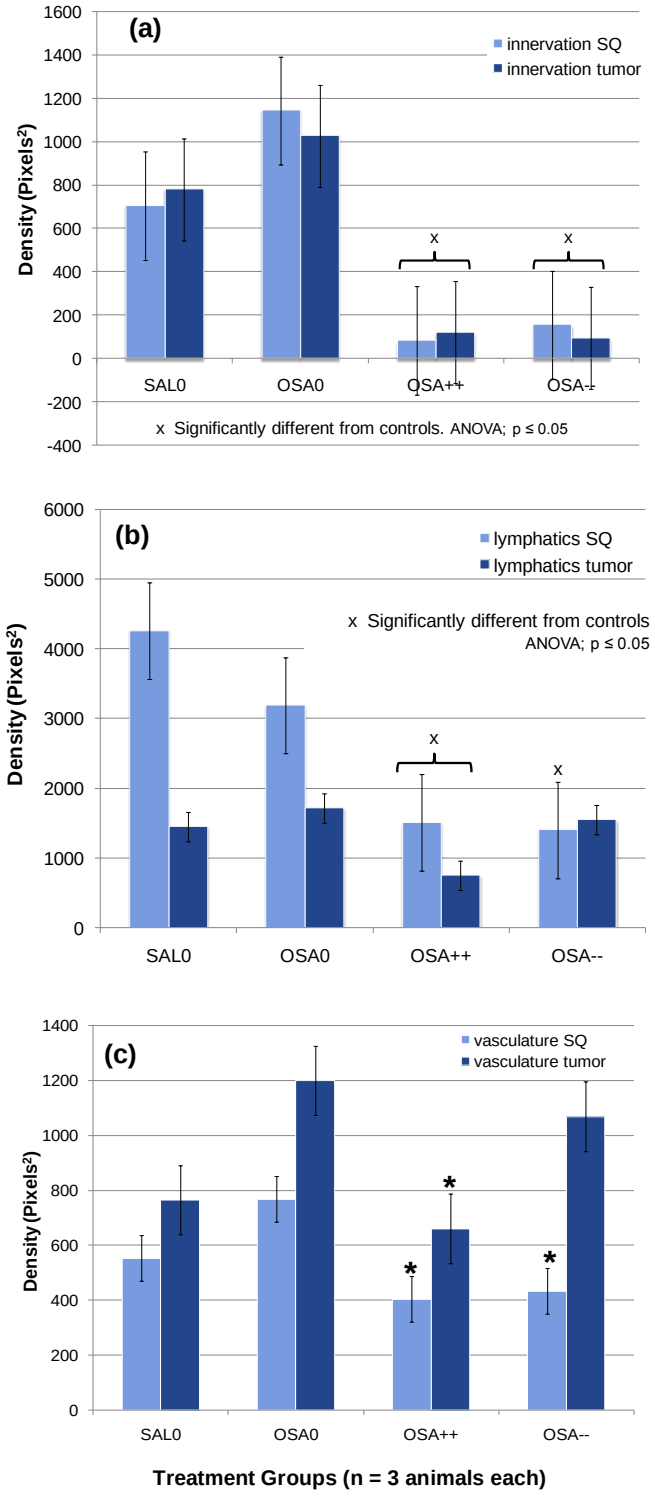

Figure 6. Quantification of **a)** nerve fibers, **b)** lymphatics, and **c)** vasculature in the areas outlined in Figure 4. ***** Significantly different from tumor (OSA∅) control. ANOVA for repeated measures with post-hoc comparison employing Bonferroni test. Unpaired Student t-test. $p \leq 0.05$ with 5% error. SQ = subcutaneous

2. Does electroacupuncture reduce nociception in mice with experimentally-induced osteosarcoma?

A. Behavioral Tests

The use of von Frey filaments is a standard technique for mechanical hypersensitivity testing (Le Bars et al., 2001). Mice were placed under spacious glass cups on a wire mesh grid and allowed to acclimate for at least 45 minutes. Mechanical hypersensitivity was tested using a variety of von Frey filaments applied through the grid to the plantar surface of each hind paw (Figure 7). Von Frey filaments were touched against the skin for a few seconds until they slightly buckled. Contact was made 10 times, and aversion behaviors (e.g. paw twitch or withdrawal, glancing towards the paw being tested) recorded as the total count out of 10. The scoring was carried out for each hind paw, starting with the right one. A baseline measure for each test was established prior to tumor injections, as well as before and after electroacupuncture application (Standard von Frey Testing Protocol utilized in the Beitz Laboratory).

Figure 7. Balb-c mouse being tested for mechanical hypersensitivity with von Frey filament (#2.83).

B. Mechanical Hyperalgesia Results

Mechanical hyperalgesia consistently increased with tumor growth, but this increase was less in mice receiving electroacupuncture, regardless of regimen.

Hyperalgesia dropped slightly on the days when electroacupuncture was performed, but rose again 24 hours later (Figure 8). One time administration of electroacupuncture delayed the development of mechanical hypersensitivity, which did not rise significantly until after Day 17 (Figure 8 a).

In spite of the astonishing tumor-inhibiting effects that were observed in OSA++ animals (Figure 3 b), twice weekly electroacupuncture treatment starting on Day 3 post implantation did not inhibit the development of mechanical hyperalgesia. This is an important observation, as it indicates that hyperalgesia may not be due solely to cancer growth, but also to the sensitization of nociceptive afferents by chemical mediators, likely released by the tumor itself.

Another indicator that the tumor might have been the source of this sensitization was the absence of any of the cardinal signs of inflammation in the heel.

Once the tumor was established (Day 7 and onward), electroacupuncture was unable to inhibit or slow the cancer growth, at all. Instead it actually had the opposite effect and potentiated the growth of the tumor. Similarly, mechanical hyperalgesia was not alleviated, but rose steeply during the second and third week of the study paralleling the increased tumor growth (Figure 8 b).

With very few exceptions, hypersensitivity scores did not differ significantly between males and females.

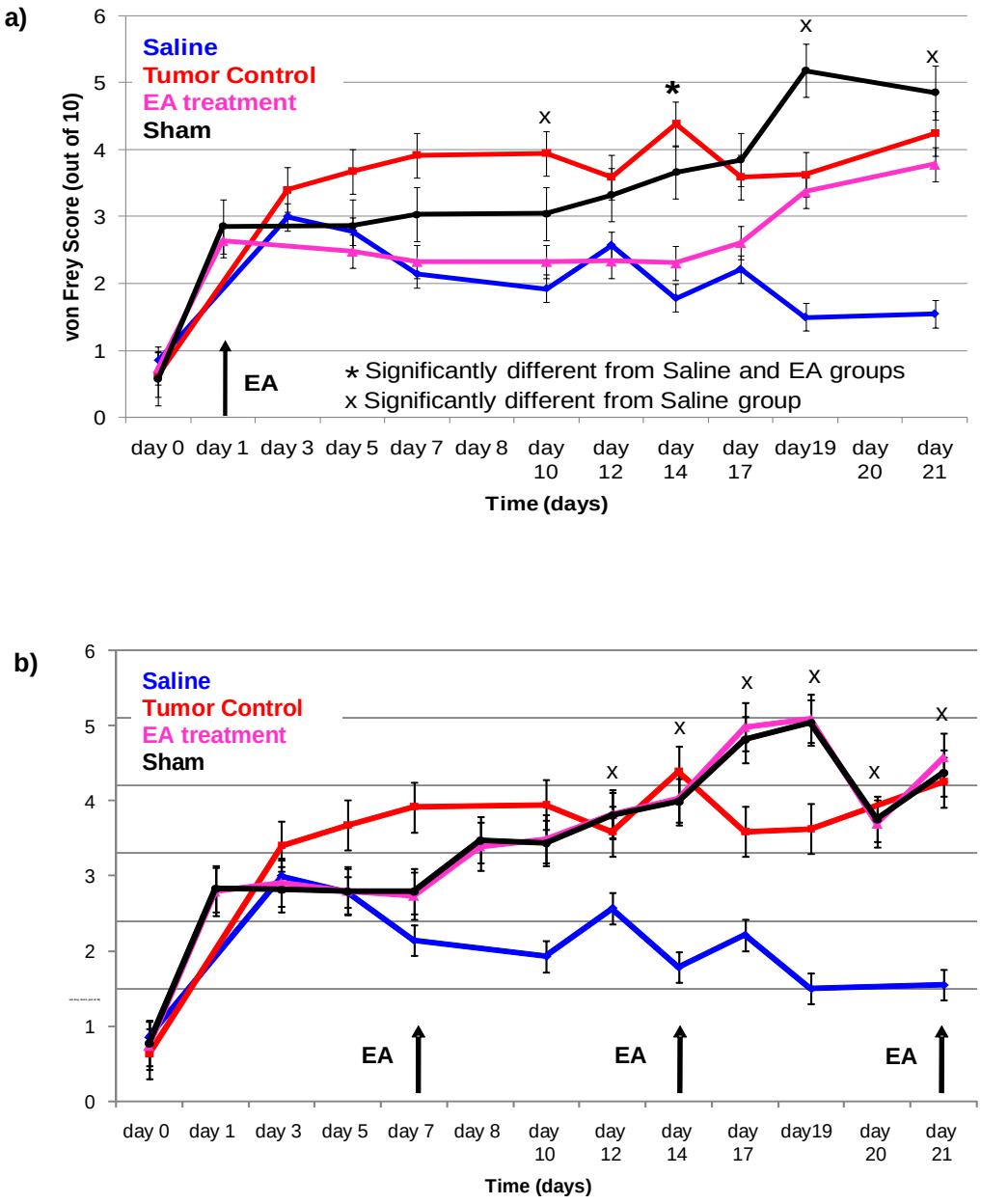

Figure 8. Mechanical hypersensitivity as measured by von Frey test. Pooled data from both male and female mice. (a) OSA+1 and OSA-1, single treatment 1 day post implantation. (b) OSA+ and OSA-, once weekly treatments for 21 days starting on Day 7 post implantation. Black arrows indicate days of electroacupuncture and sham treatments. ANOVA for repeated measures with post-hoc comparison employing Bonferroni test. Unpaired Student t-test. $p \leq 0.05$ with 5% error.

C. c-fos Immunohistochemistry

The harvesting of spinal cords and subsequent c-fos immunohistochemistry techniques also have been used extensively in our lab over the past 17 years and are described for rats by Lee and Beitz (1992) and Beitz et al. (2004). Furthermore, c-fos expression in the spinal cord and brain has been successfully correlated to pain transmission and to acupuncture activation and this allows areas involved in pain or acupuncture to be mapped out in the Central Nervous System (CNS) (Lee and Beitz, 1992 and 1993; Harris, 1998).

Following is the standard c-fos immunohistochemistry protocol using the Avidin-Biotin-Peroxidase Method[24], as utilized by the Beitz laboratory. Spinal cords were removed from spinal columns harvested after transcardial perfusion and refrigerated in 30% sucrose in PBS by carefully cracking and peeling off the thoracic and lumbar vertebrae under the dissecting microscope. The lumbosacral enlargement of the harvested spinal cords was identified, removed and bisected approximately at the level of L4. Both lumbosacral pieces were mounted on a sliding microtome equipped with a freeze stage, and once they were frozen, the right ventral horn was carefully notched for identification using a 30 G needle.

After this, 20 40-micron-thick sections comprising L4 and L5 spinal segments were cut transversely from the bisected spinal pieces (40 sections per mouse to allow for accidental damage, spillage, etc.). The cut tissue was collected in small baskets submerged in PBS. Baskets containing the free-floating cut sections were transferred to a blocking solution (2% goat serum in 0.3% TX/PBS) for 1 hour at room temperature. From there, they were transferred directly to primary antibody (c-fos diluted 1:2500 in blocking solution) and incubated on an orbital shaker overnight at 4°C.

The next day, the tissue underwent 5 x 5-minute rinses with PBS, before being transferred to biotinylated secondary antibody (goat anti-rabbit IgG diluted 1:250 in blocking solution), before being incubated on an orbital shaker at room

[24] Vectastain Elite ABC-peroxidase kit, by Vector Laboratories, Burlingame, CA

temperature for 2 hours. Afterwards, tissue sections were rinsed 5 x 5 minutes in PBS and incubated in the avidin-biotin complex (ABC) solution for 1 hour at room temperature on the orbital shaker. Three 5-minute rinses in PBS are next, followed by 2 x 5-minute rinses in Tris buffer.

Finally, the tissue is incubated in a DAB (diaminobenzidine tetrahydrochloride) solution until it turns a uniform medium brown color, and rinsed 5 times in PBS for a final time.

To mount, all 40 tissue sections are floated in Petri dish containing equal parts PBS and distilled water. Under the dissection microscope, the each section was inspected and mounted on gel-coated slides using a fine paint brush. After rinsing off any salt residues, slides were dehydrated for 5 minutes each in 70% and 95% ethanol, and for 10 min in 100% ethanol. After a 5-minute, slides were cleaned in xylenes, coverslipped with DPX mounting medium and allowed to dry completely.

The ten best-looking sections with the greatest number of c-fos immunoreactive neurons in laminae I-VI were selected from each animal and analyzed using the Metamorph® cell counting program.

D. c-fos Expression Results

Analysis of spinal c-fos expression revealed noticeable variety between the treatment groups. One interesting development was the reduced c-fos expression seen in female tumor control animals compared to males, even though females experienced a higher percentage of tumor growth. Both males and females had significantly higher c-fos expression in the left dorsal horn of the spinal cord, ipsilateral to the implanted tumor and contralateral to the needle placement and electrical stimulation.

Both early treatments (OSA+1 and OSA++) significantly reduced c-fos expression in both males and females (Figures 10a and b), whereas late

treatment (OSA+) was associated with significantly increased c-fos expression (Figure 9).

It appears that osteosarcoma-induced nociception does result in significant c-fos expression in the spinal cord for at least 3 weeks post implantation and electroacupuncture has a significant effect on the osteosarcoma-induced expression.

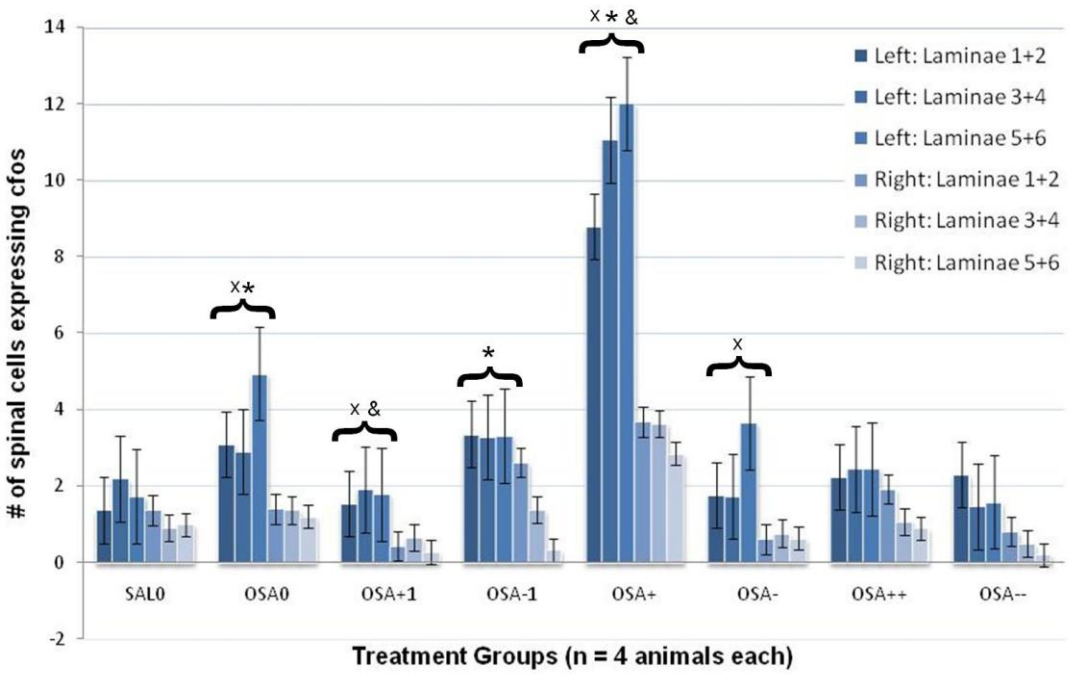

Figure 9. Mean number of cells expressing cfos in the spinal cord in the L4 – L5 segments. Pooled data from both males and females. Data has been divided into laminae 1-6 on both the left and right sides.

* Significantly different from SALØ group. & Significantly different from OSAØ group. x Significantly different from right side. ANOVA; $p \leq 0.05$

3. Does gender influence electroacupuncture effects on tumor growth and nociception in mice with experimentally-induced osteosarcoma?

A. Gender differences

All measurements taken during the study duration were statistically compared between the sexes.

Vaginal lavage with 10-20 μL of normal saline was carried out using a small tipped pipette while female mice were still anesthetized following electroacupuncture treatment. The fluid was immediately transferred to a microscopic slide, stained with a drop of methylene blue, and coverslipped. Vaginal smears were then used to differentiate between the four stages of the estrous cycle: diestrus, proestrus, estrus, and metestrus (Rugh, 1990).

B. Results

With few exceptions, there were no significant gender differences in tumor growth or von Frey scores that correlated with stages of the estrous cycle. Estrous cycles in females varied greatly among female mice and were not synchronized within groups. They showed no correlation to electroacupuncture or behavioral testing, indicating no direct hormonal influence.

7. DISCUSSION

1. Does electroacupuncture affect tumor growth in mice with experimentally-induced osteosarcoma?

a) Does electroacupuncture have a tumor-enhancing or tumor-inhibiting effect and is this dependent on specific electroacupuncture treatment strategies?

The research presented documents the effects of electroacupuncture on osteosarcoma growth in the calcaneus bone in mice. There have been no previous studies evaluating the effects of acupuncture on osteosarcoma tumor growth and thus these results are novel and provide insights into the effects that electroacupuncture has on tumor growth and tumor nociception

The average tumor take was 81.46% (i.e. tumors grew in 8/10 mice that were implanted with K7M2 cells), which was lower than previously reported. Khanna et al., (2000) reported a tumor take of 95% for their K7M2 cell line. Their orthotopic model, however, involved implantation of actual tumor tissue into the tibia, which means that the cells implanted already had established themselves in previous tissue, and, therefore were primed for optimum invasiveness. In addition, Khanna's group implanted 5 times more cells (in 10 times as much volume) than we did in our experiments, which probably explains the differences in tumor take, as well as some of the differences in tumor metastasis between their studies and ours.

The K7M2 osteosarcoma cells used are invasive and metastasize at a rapid rate (Khanna et al., 2000). Despite the use of aseptic protocols, infections are known to occur, and may have affected the general health and tumor growth post implantation. Conversely, tumors would not grow at all in some mice, depending on cell viability, individual immunity, or human error during

implantation. This assessment was based on the Beitz lab's experience with the hind paw tumor model, which has been used regularly in the laboratory since 1999 with a number of different cancer cell lines. Occasionally, difficulties were encountered when culturing the K7M2 cell line. This was usually attributable to culture contamination or to poor handling of the cells and was overcome by growing a different lot of frozen cells and assuring that the cells, culture room and hood were free from bacterial contaminants.

Nonetheless, our tumor model of injecting tumor cells into the calcaneus bone has performed exceedingly well with this specific cell line. Even though the model has been used in the Beitz lab for more than 10 years in variety of cancer and pain studies (Wacnik et al., 2000; Wacnik et al., 2001), it has never been employed in association with electroacupuncture and osteosarcoma. Despite the lower tumor take observed, our model had the advantage over femoral or humeral implants, in that tumors could be verified visually a week earlier than in the model developed by Khanna et al. (2000), and tumor growth could be followed easily using external measurements. Furthermore, the location of the tumor in the calcaneus was particularly convenient, as it provided ready access for the measurement of primary hyperalgesia, which is more difficult to measure in bone tumors involving the femur or tibia.

In most cases, the primary tumor stayed in the heel, where it was readily visible as early as 7 days post implantation. Frequently, however, the tumor was not well defined and had spread to involve the entire foot. On some occasions, the tumor had travelled up the hind leg and settled in the tibia or even the thigh. It is possible that since the K7M2 cell line was developed in tibial tissue, the cells had some internal homing mechanism that caused them to migrate towards the tibia (Abu-Amera, 2009).

It was surprising that tumors seemed to grow better in females than in males (86.52% tumor take versus 76.40%, respectively), which was especially noticeable in tumor control groups and groups experiencing enhanced tumor growth. This stands against the common consensus that males are more

predisposed to develop osteosarcoma than females (Theilen and Madewell, 1987; Pool, 1990; Ries et al., 1999).

Osteosarcoma frequently originates at or near the bone metaphyses, which contain bone-forming mesenchymal stem cells (Kramarova and Stiller, 1996), and is thus considered to be a malignant tumor of osteoblasts (Sanerkin, 1980). Fohr et al. (2000) observed that even though estrogen and testosterone can have different effects on different tumor cell lines, the response to hormonal treatment with sex steroids was not related to the gender of the osteosarcoma cell line, but rather depended on its osteoblastic commitment. This supports the idea that differences in bone structure or metabolism may be responsible for differences in tumor take.

One possible explanation for the observed gender differences may be bone density (Kung, 2008). Osteosarcoma preferentially originates from the metaphyseal portions of bones in children and young adolescents, whose bones have not yet fully calcified (Miller et al., 1996). The animals used for this thesis project were young adult mice, and during tumor implantations, it was noticeably easier to drill into the heels of females, the bones of which appeared to be softer than those of the males, implying a lower degree of mineralization.

Moreover, since osteosarcoma has been associated with trauma and subsequent healing and repair (Operskalski et al., 1987), it is possible that gender differences in bone healing may be responsible for the differences seen in our study. In female rats, sex-specific differences in bone healing, as indicated by a compromised mechanical competence of the callus in females compared with males, have been attributed to a decrease in the quantity of mesenchymal stem cells (Strube et al., 2009).

Alternatively, recent evidence suggests that osteoblastic estrogen receptors are important to the maintenance of normal bone density (Ikeda et al., 1993; Hoshino et al., 1995), and both acupuncture and electroacupuncture increase serum estrogen levels, which, in turn, increase the metabolism of osteoblasts to maintain bone density in cases of osteoporosis (Wei et al., 2007;

Ma et al., 2008). This increased estrogen-dependent mechanism may be another explanation why the tumor take in this study was higher in the female than the male mice.

Thus, there are a number of reasons that could explain the difference in tumor take and the differences in male/female tumor growth observed in the present study. However, the effects of acupuncture on tumor growth were similar regardless of sex. Indeed, tumor growth patterns were comparable between males and females, without any significant differences. With the exception of OSA++, osteosarcoma growth increased between Day 10 and Day 12 in all implanted animals, regardless of treatment. Tumor growth was significantly reduced in all mice receiving electroacupuncture treatment twice weekly starting on Day 3 or Day 5 (OSA++ and OSA5+). This indicates that early and frequent treatment interferes with tumor growth. The most striking tumor growth – more than 100% for most – occurred in mice receiving once weekly acupuncture treatments starting on Day 7 (OSA+) or Day 14 (OSA7+) post implantation. This implies that electroacupuncture was ineffective in inhibiting the tumor once it was established and rather served to increase tumor growth.

Anecdotal accounts of acupuncture and electroacupuncture imply that needling on or near the tumor exaggerates its growth. The results of this research indicate that this is not necessarily the case. In fact, the onset and frequency of treatment appear to greatly influence whether electroacupuncture has inhibitory or tumor-enhancing effects. Diverse acupuncture modes and manipulations, such as intensity, frequency, duration and interval between stimulations, will activate different mechanisms and thus affect different responses (Langevin and Yandow, 2002; Lund and Lundeberg, 2008; Linde et al., 2009). Furthermore, the results presented are based on electroacupuncture stimulation (4 Hz, 7 V) delivered at the contralateral ST-36. Whether similar results can be produced using other acupuncture points, either alone or in combination with others, remains to be investigated.

b) Does electroacupuncture cause changes in the innervation and vascularization of osteosarcoma tumors in a way that could impact tumor growth?

Tumor sections scanned with Laser Scanning Confocal Microscopy showed clear structural changes at 4x magnification, which were even more obvious at 20x. Animals in treatment groups characterized by increased tumor growth or size (such as OSA+ or OSA7+) had more defined tumors or more extensive destruction of normal tissue.

Hyperalgesic mice with bone tumors, such as fibrosarcomas, exhibit increased innervation and reduced vascularization (Wacnik et al., 2005). The results of this research, however, showed that innervation and vasculature in the osteosarcoma tumor and surrounding tissue did not differ significantly between saline and tumor control groups. Early and frequent electroacupuncture and non-stimulated needling significantly decreased both subcutaneous and tumor innervation. Interestingly, OSA-, OSA5-, OSA7+, and both prophylactic treatment groups, also exhibited reduced innervation. This indicates that frequency of treatment may be a determining factor, in addition to early interference with tumor growth or establishment.

The functional properties of the tumor vasculature are especially important in metastasis and tumor progression (Bacci et al., 1998; Harris et al., 1998; Hansen-Algenstaedt et al., 2005; Fuhrhop et al., 2009), and in producing a heterogeneous metabolic microenvironment, which contributes to genetic instability and inefficiency of tumor therapies (Hansen-Algenstaedt et al., 2005; Fuhrhop et al., 2009).

The density of both subcutaneous and tumor vasculature did not change significantly regardless of treatment, and remained similar to both controls. This agrees with observations in a mouse model of breast cancer growth in bone. The total functional vascular density remained unaltered, despite a significant loss in small vessels and a concomitant increase in vascular diameter (Fuhrhop et al., 2009). The most abundant vessels were the subcutaneous lymphatics, which

also remained comparable to controls for most treatments throughout the study. However it is important to note that both OSA++ and OSA+ treated mice, as well as OSA7- and the two prophylactic treatments, did have significantly fewer lymph vessels. Tumor cell implantation initially causes a significant increase in the permeability of pre-existing vessels (Hansen-Algenstaedt et al., 2005), and manual acupuncture has been found effective in controlling the edematogenic response due to capsaicin-induced edema in a rat model of experimentally-induced inflammation (Ceccherelli et al., 1996). Thus, electroacupuncture may have a more pronounced effect during the early stages of tumor growth because it affects the permeability of blood vessels. Recent work by Edwards and coworkers (2008) has shown that in normal bone, lymphatic vessels are not identified in cortical or cancellous bone but are seen in connective tissue overlying the periosteum. With the exception of lymphangioma, primary benign and malignant bone tumors (as well as secondary carcinomas) that were confined to bone did not contain lymphatic vessels. However, primary and secondary bone tumors that had extended through the bone cortex contained lymphatic vessels that seemed to extend for a short distance from surrounding soft tissues into the tumor. In particular these investigators showed that in three cases of osteosarcoma that had extended through the bone cortex and had lymph node metastases, each of these tumors were found to contain lymphatic vessels within the tumor. Our results are consistent with those of Edwards et al, but futher show that tumor lymphatics are somewhat plastic in nature and thus their density can be influenced by electroacupuncture stimulation. Tumors produce excess fluid that continually percolates from the tumor towards nearby lymphatic vessels. Tumor cells use a clever chemical strategy to exploit this slow, one-way flow in order to migrate to functional lymphatic vessels and ultimately metastasize. If acupuncture reduces the number of lymphatic vessels at the tumor site, this should reduce both lymphatic drainage and metastatic tumor cell movement along lymphatics. While we were unable to confirm that acupuncture reduced tumor metastasis in our study, it is likely that it does so via a reduction in lymphatics at the tumor site.

Taken together, these reductions in innervation and vascularization imply that early electroacupuncture may interfere with the tumor's ability to set up a vascular network for nourishment and communication with the rest of the body (Ta et al., 2009), while at later stages, the tumor may destroy the structures that maintain it. This supports previous observations that the efficiency of adjuvant therapies depends on the timing of their initiation, most likely because the morphological and functional properties of tumor vasculature vary from tumor onset to late-stage disease (Hansen-Algenstaedt et al., 2005).

c) Does electroacupuncture alter tumor metastasis to the lungs?

Even though many of the harvested lungs exhibited distinct areas of white or grey discoloration involving parts of or complete lung lobes, histopathological findings were inconclusive. It is likely that the 21-day study period was too short for metastases to develop. Khanna et al. (2000) did not detect tumors until the second week of implantation, and achieved target tumor size (in the tibia) of 450 mm^3 20 – 30 days post implantation. Therefore, it was a reasonable expectation to set the study end point at 21 days.

Based on previous experiences with our fibrosarcoma model, it was possible that some animals would not reach the day-21 study endpoint due to advanced tumor growth. It is this author's experience that by the time a tumor reaches 8 – 10 mm in diameter, it frequently begins to disrupt the integrity of the skin and cause deformity of the foot due to pathological fractures and excessive tissue accumulation. This is a common occurrence with bone cancers (Mantyh et al., 2002; Delaney et al., 2008; Colvin and Fallon, 2008). As soon as a tumor reaches this destructive stage, RAR and IACUC protocols require euthanasia of the animal to prevent any further suffering. Thus, the final tumor size was one factor that limited the study period.

Another factor that may be responsible for the results obtained was that in this experiment, the lungs used had been fixed in 4% paraformaldehyde, while

Khanna et al., (2000) stained freshly harvested lungs. This could have led to inadequate or incomplete staining and the inconclusive results observed. However, the nature of the electroacupuncture study made it impossible for lungs to be harvested fresh, without getting fixed first.

However, considering the importance of the tumor vasculature in metastasis and tumor progression (Bacci et al., 1998; Harris et al., 1998; Hansen-Algenstaedt et al., 2005; Fuhrhop et al., 2009), and the documented effect of acupuncture on vascular permeability (Ceccherelli et al., 1996), which is changed following tumor implantation (Hansen-Algenstaedt et al., 2005), the development of metastases in the lungs should have been significantly reduced, if not prevented.

A follow-up project should be done using labeled cancer cells, which could easily be tracked and identified in the lungs, without having to wait for grossly visible metastatic nodules to form. However, care should be taken, as some label and stains can slow or inhibit the growth and function of cells maintained in culture (Dass and Choong, 2007). Alternatively, tumor cell apoptosis in both, the periphery and the lungs, could be quantified using the TUNEL assay (Klenke et al., 2006).

2. Does electroacupuncture reduce nociception in mice with experimentally-induced osteosarcoma?

Mechanical hyperalgesia consistently increased with tumor growth, but this increase was less in mice receiving electroacupuncture, regardless of regimen. Hyperalgesia dropped slightly on the days when electroacupuncture was performed, but rose again 24 hours later. This is in support of previously published studies describing a transient analgesic state (Huang et al., 2002).

One time administration of electroacupuncture delayed the development of mechanical hypersensitivity, which did not rise significantly until after Day 17.

Once cancer infiltrates the musculo-skeletal system, development of persistent somatic pain is inevitable (Viganó et al., 1998; Walsh, 2005, Mantyh et al., 2002). However, in spite of the astonishing tumor-inhibiting effects that were observed in OSA++ animals, twice weekly electroacupuncture treatment starting on Day 3 post implantation did not inhibit the development of mechanical hyperalgesia. This is an important observation, as it indicates that hyperalgesia may not be due solely to tumor growth and tumor size, but also to the sensitization of nociceptive afferents by chemical mediators, likely released by the tumor itself (McMahon, 1996; Wacnik et al., 2001; Mantyh et al., 2002; Sabino et al., 2002; Sevcik et al., 2005; Wacnik et al., 2005a).

It is possible that this sensitization of afferent nociceptors by tumor-generated pro-inflammatory substances was responsible for the response to electroacupuncture seen, as inflammation was found to facilitate so-called 'responders' (Sekido et al., 2003).

Once the tumor was established (Day 7 and onward), electroacupuncture was unable to inhibit or slow the cancer growth, at all. Instead it appeared to potentiate the growth of the tumor. Similarly, mechanical hyperalgesia was not alleviated, but rose steeply during the second and third week of the study. With very few exceptions, hypersensitivity scores did not differ significantly between males and females. This raises important questions regarding the use of acupuncture to treat tumor pain in human or animal medicine, if the pain relieving treatment actually increases tumor growth and pain sensitivity.

Electroacupuncture readily induces c-fos in the spinal cord and various brain regions, suggesting that transcription factors are also involved in processing acupuncture signals (Ji et al., 1993a; Ji et al., 1993b; Lee and Beitz, 1993; Guo et al., 1996). Analysis of spinal c-fos expression revealed noticeable variety among the treatment groups. One interesting development was the reduced c-fos expression seen in female tumor control animals compared to males, even though females experienced a higher percentage of tumor growth. Women do exhibit lower pain thresholds than men (Berkley, 1997; Fillingim and

Ness, 2000; Craft et al., 2004), so one would expect them to also have increased c-fos expression. And, yet, c-fos expression was similar in both males and females. Both demonstrated significantly higher c-fos expression in the left dorsal horn of the spinal cord, ipsilateral to the implanted tumor and contralateral to the needle placement and electrical stimulation. Early treatments (OSA+1 and OSA++) significantly reduced c-fos expression in both males and females, whereas late treatment was associated with significantly increased c-fos expression, which paralleled the increase in tumor growth produced by late acupuncture treatment.

It appears, therefore, that osteosarcoma-induced nociception does result in significant c-fos expression in the spinal cord for at least 3 weeks post implantation and electroacupuncture has a significant effect on the osteosarcoma-induced expression.

3. Does gender influence electroacupuncture effects on tumor growth and nociception in mice with experimentally-induced osteosarcoma?

With few exceptions, there were no significant gender differences in tumor growth or von Frey scores that correlated with stages of the estrous cycle. Estrous cycles in females varied greatly among female mice and were not synchronized within groups. They showed no correlation to electroacupuncture or behavioral testing, indicating no direct hormonal influence. Even though acupuncture and electroacupuncture are known to increase serum estrogen levels (Wei et al., 2007; Ma et al., 2008), these increases were not high enough to affect the estrous cycle in this study. Furthermore, testosterone has been shown previously to play no role in electroacupuncture-induced analgesia (Kong et al., 1991).

Taken together, and apart from any individual genetic differences (Mogil, 1999; Chae et al., 2006), electroacupuncture treatment had no discernable gender-related effects.

Conclusion

In summary, electroacupuncture can have tumor-inhibiting or tumor-enhancing effects, depending on when and how frequently it is applied. With few exceptions, electroacupuncture reduced tumor innervation and lymphatics in animals receiving early, frequent electroacupuncture. However, blood vessel density and distribution remained mostly unaffected. This implies that electroacupuncture exerts its effects on tumor growth in part by reducing innervation, which in turn may alter the tumor's ability to secrete chemical mediators and pro-inflammatory substances. Acupuncture has also been shown in other studies to reduce inflammation and it seems likely that it may also reduce tumor growth by reducing tumor associated inflammation.

Electroacupuncture exerts a transient analgesic effect on animals with osteosarcoma, and, therefore, cannot be endorsed as a stand-alone therapy for pain management. However, due to the many mechanisms by which it can exert an anti-hyperalgesic effect, it represents an ideal complementary therapy to be included in a multimodal pain management regimen.

Furthermore, electroacupuncture lends itself to many applications as a novel study tool. It is easily incorporated in a number of laboratory or clinical environments and research setups, and provides an innovative way to study the mechanisms underlying tumor growth and metastasis.

Future studies are required to test other acupuncture points alone and in combination as to their ability to interfere with cancer growth. Different stimulation intensities, as well as unilateral versus bilateral stimulation should also be evaluated. In addition, given the many mechanisms involved in acupuncture and electroacupuncture analgesia, it is vital to identify exactly which ones are involved in tumor anti-nociception. It is my firm belief that this study holds importance for future clinical and mechanistic studies of cancer and nociception. Improvements in study design for future investigations, such as better sham control conditions and a longer study period, may confer greater sensitivity to detect true therapeutic effects of electroacupuncture.

8. REFERENCES

Aanonsen LM, Lei S, Wilcox GL (1990) Excitatory amino acid receptors and nociceptive neurotransmission in rat spinal cord. Pain **41**, 309–321

Abdi S, Lee DH, Chung JM (1998) The anti-allodynic effects of amitriptyline, gabapentin, and lidocaine in a rat model of neuropathic pain. Anesth Analg **87,** 1360-1366

Abu-Amera Y (2009) Inflammation, cancer, and bone loss. Curr Opin Pharm **9:4**, 427-433

Akiyama T, Dass CR, Choong PF (2008) Novel therapeutic strategy for osteosarcoma targeting osteoclast differentiation, bone-resorbing activity, and apoptosis pathway. Mol Cancer Ther **7:11**, 3461-3469

Ali J, Yaffe CS, Serrette C (1981) The effect of transcutaneous electric nerve stimulation on postoperative pain and pulmonary function. Surgery **89:4**, 507-512

Alkhalaf M, Jaffal S (2006) Potent antiproliferative effects of resveratrol on human osteosarcoma SJSA1 cells: Novel cellular mechanisms involving the ERKs/p53 cascade. Free Radic Biol Med **41:2**, 318-325

Allen C (2004) Animal Pain. Nous **38**, 617-643

AL-Nasrallah LJ, Al-Ahmed AH, Saeed MAM (2007) "The Pattern of Osteosarcoma in Southern Part of Iraq". Basrah Journal Original Article. Available at http://www.basmedcol.com/Mubder%202007.pdf. Accessed on 6/10/2009.

American Cancer Society, Inc. "The History of Cancer". Available at: http://www.cancer.org/docroot/CRI/content/CRI_2_6x_the_history_of_cancer_72asp?sitearea=CRI. Accessed June 10, 2009

Anderson PM, Katsanis E, Leonard AS, et al. (1990) Increased local antitumer effects of interleukin 2 liposomes in mice with MCA-106 sarcoma pulmonary metastases. Cancer Res **50:6,** 1853-1856

Andersson SA, Holmgren E (1975) On acupuncture analgesia and the mechanism of pain. Am J Chin Med **3**, 311–334

Arthur HM, Patterson C, Stone JA. The role of complementary and alternative therapies in cardiac rehabilitation: a systematic evaluation. Eur J Cardiovasc Prev Rehabil **13:1,** 3-9

Asham E, Shankar A, Loizidou M, et al. (2001) Increased endothelin-1 in colorectal cancer and reduction of tumour growth by ET(A) receptor antagonism. Br J Cancer **85**, 1759–1763

Authier N, Balayssac D, Marchand F, et al. (2009) Animal models of chemotherapy-evoked painful peripheral neuropathies. Neurotherapeutics **6:4**, 620-629

Baamonde A, Lastra A, Fresno MF, et al. (2004) Implantation of tumoral XC cells induces chronic, endothelin-dependent, thermal hyperalgesia in mice. Cell Mol Neurobiol **24**, 269–281

Bacci G, Briccoli A, Ferrari S, et al. (1993) Osteosarcoma of the extremities metastatic at presentation. Results obtained with primary chemotherapy followed by simultaneous resection of the primary and metastatic lesion [Article in Italian, English abstract]. Minerva Chir **48:1-2**, 35-45

Bacci G, Briccoli A, Mercuri M, et al. (1998) Osteosarcoma of the extremities with synchronous lung metastases: long-term results in 44 patients treated with neoadjuvant chemotherapy. J Chemother **10:1,** 69-76

Bai B, Wang H, Liu WY, et al. (1999) Effect of anti-opioid peptide sera on the enhancement of electroacupuncture analgesia induced by neurotensin in PAG of rats [Article in Chinese, English abstract]. Acta Physiol Sin **51**, 224–228

Baillie JK, Power I (2006) The mechanism of action of gabapentin in neuropathic pain. Curr Opin Invest Drugs **7:1**, 33-39

Ballou LR, Botting RM, Goorha S, et al. (2000) Nociception in cyclooxygenase isozyme-deficient mice. Proc Natl Acad Sci USA **97**, 10272-10276

Bardet JF, Weisbrode S, DeHoff WD (1989) Extraskeletal osteosarcoma in 2 dogs. JAVMA **194**, 1452-1456

Barger A, Graca R, Bailey K, et al. (2005) Use of alkaline phosphatase staining to differentiate canine osteosarcoma from other vimentin-positive tumors. Vet Pathol **42**, 161-165

Basbaum AI, Clanton CH, Fields HL (1976) Opiate and stimulus-produced analgesia: functional anatomy of a medullospinal pathway. Proc Natl Acad Sci USA **73:12,** 4685-4688

Basbaum AI, Jessell TM: The perception of pain. In: Kandel ER, Schwartz JH, Jessell TM (eds.) Principles of Neural Science. McGraw-Hall, 2001, pp. 472–491

Basbaum AI, Marley N, O'Keefe J, et al. (1977) Reversal of morphine and stimulus produced analgesia by subtotal spinal cord lesions. Pain **3**, 43-56

Bassin EB, Wypij D, Davis RB, et al. (2006) Age-specific fluoride exposure in drinking water and osteosarcoma (United States). Cancer Causes Control **17:4**, 421-428

Bausell RB, Lao L, Bergman S, et al. (2005) Is acupuncture analgesia an expectancy effect? Preliminary evidence based on participants' perceived assignments in two placebo-controlled trials. Eval Health Prof **28**, 9–26

Beecher HK (1946) Pain in Men Wounded in Battle. Ann Surg **123:1**, 96-105

Beecher HK (1956) Relationship of significance of wound to pain experienced. J Am Med Assoc **161:17**, 1609-13

Beissner F and Henke C (2009) Methodological Problems in fMRI Studies on Acupuncture: A Critical Review With Special Emphasis on Visual and Auditory Cortex Activations. eCAM Advance Access published **September 30**, eCAM 2009; Page 1 of 7 doi:101093/ecam/nep154

Beitz AJ, Neman A, Shepard M, et al. (2004) A new rodent model of hind limb penetrating wound injury characterized by continuous primary and secondary hyperalgesia. J Pain **5:1**, 26-37

Belito WV, Patnaik AK, Schrader SC, et al. (1987) Osteosarcoma in cats: 22 cases (1974 – 1984). JAVMA **190:1,** 91-93

Ben-Arye E, Karkabi S, Shapira C, et al. (2009) Complementary medicine in the primary care setting: Results of a survey of gender and cultural patterns in Israel. Gend Med **6:2,** 384-97

Benedetti F (1996) The opposite effects of the opiate antagonist naloxone and the cholevyctokinin antagonist proglumide on placebo analgesia. Pain **64**, 535–543

Benedetti F, Amanzio M (1997) The neurobiology of placebo analgesia: from endogenous opioids to cholecyctokinin. Prog Neurobiol **51**, 109–125

Benedetti F, Pollo A, Colloca L (2007) Opioid-mediated placebo responses boost pain endurance and physical performance: is it doping in sport competitions? J Neurosci **27:44,** 11934-11939

Berenson J, Lichtenstein A, Porter L, et al. (1996) Efficacy of pamidronate in reducing skeletal events in patients with advanced multiple myeloma. N Engl J Med **334:8,** 488-493

Berg J, Weinstein MJ, Schelling SH, et al. (1992) Treatment of dogs with osteosarcoma by administration of cisplatin after amputation or limb-sparing surgery: 22 cases (1987-1990). J Am Vet Med Assoc **200:12**, 2005-2008

Berkley K (1997) Sex differences in pain. Behav Brain Sci **20,** 371-380

Besson J. The neurobiology of pain. Lancet **353:9164,** 1610-1615

Betourne A, Familiades J, Lacassagne L, et al. (2008) Decreased motivational properties of morphine in mouse models of cancerous- or inflammatory-chronic pain: implication of supraspinal neuropeptide FF(2) receptors**.** Neuroscience **157:1,** 12-21

Beuth J, Schierholz JM (2007) Review. Evidence-based complementary oncology. Innovative approaches to optimize standard therapy strategies. In Vivo **21:2**, 423-428

Biella G, Sotgiu ML, Pellegata G, et al. (2001) Acupuncture produces central activations in pain regions. Neuroimage **14**, 60–66

Bing Z, Cesselin F, Bourgoin S, et al. (1991a) Acupuncture-like stimulation induces a heterosegmental release of Met-enkephalin-like material in the rat spinal cord. Pain **47,** 71–77

Bing Z, Villanueva L, Le Bars D (1990) Acupuncture and diffuse noxious inhibitory controls: naloxone-reversible depression of activities of trigeminal convergent neurons. Neuroscience **37**, 809–818

Bing Z, Villanueva L, Le Bars D (1991b) Acupuncture-evoked responses of subnucleus reticularis dorsalis neurons in the rat medulla. Neuroscience **44,** 693–703

Blumenthal DT (2009) Assessment of neuropathic pain in cancer patients. Curr Pain Headache Rep **13:4**, 282-287

Bogduk N (2002) The Physiology of Deep, Somatic Pain. Australasian Musculoskeletal Medicine **7:1,** 6-15

Boivie J: Central pain syndromes. In: Campell JN (ed.) Pain - An Updated Review. Seattle: IASP Press, 1996, p. 23-29

Bossut DFB, Page EH, Stromberg MW (1984) Production of cutaneous analgesia by electro-acupuncture in horses: variations dependent on sex of subject and locus of stimulation. Am J Vet Res **4**, 620-625

Boucher TJ, Okuse K, Bennett DL, et al. (2000) Potent analgesic effects of GDNF in neuropathic pain states. Science **290**, 124–127

Brainin-Mattos J, Smith ND, Malkmus S, et al. (2006) Cancer-related bone pain is attenuated by a systemically available delta-opioid receptor agonist. Pain **122:1-2,** 174-181

Breivik H, Cherny N, Collett B, et al. (2009) Cancer-related pain: a pan-European survey of prevalence, treatment, and patient attitudes. Ann Oncol **20:8,** 1420-1433

Brescia F, Portenoy R, Ryan M, et al. (1992) Pain, opioid use, and survival in hospitalized patients with advanced cancer. J Clin Oncol **10:1**, 149-155

Bridges D, Thompson SWN (2001) Mechanisms of neuropathic pain. Br J Anaesthesia **87**, 21-26

Briggs E (2008) Cultural perspectives on pain management. J Perioper Pract **18:11,** 468-471

Brown ML, Ulett GA, Stern JA (1974) Acupuncture loci: techniques for location. Am J Chin Med **2**, 67-74

Bucher JR, Hejtmancik MR, Toft JD, et al. (1991) Results and conclusions of the National Toxicology Program's rodent carcinogenicity studies with sodium fluoride. Int J Cancer **48:5**, 733–737

Buckley JD, Pendergrass TW, Buckley CM, et al. (1998) Epidemiology of osteosarcoma and Ewing's sarcoma in childhood: a study of 305 cases by the Children's Cancer Group. Cancer **83:7**, 1440-8

Buesa I, Urrutia A, Aira Z, et al. (2008) Depression of C fibre-evoked spinal field potentials by the spinal delta opioid receptor is enhanced in the spinal nerve ligation model of neuropathic pain: involvement of the mu-subtype. Neuropharmacology **55:8**, 1376-1382

Busko M (2009) "Acupuncture Has Minimal Analgesic Effect Over Sham Acupuncture". Medscape Medical News. Available at http://www.medscape.com/viewarticle/588572?src=rss. Accessed June 10, 2009

Cabioğlu MT, Cetin BE (2008) Acupuncture and Immunomodulation. Am J Chin Med Vol **36:1**, 25–36

Campbell A (1999) Acupuncture: Where to Place the Needles and for How Long. Acupuncture in Medicine **17:2**, 113-117

Campbell JN, Meyer RA (2006) Mechanisms of neuropathic pain. Neuron **52:1**, 77-92

Cao W, Deng Y, Dong X, et al. (1993) Effects of electroacupuncture at different frequencies on the nociceptive response and central contents of GABA and glutamic acid in arthritic rats. [Article in Chinese, English abstract]. Zhen Ci Yan Jiu **18:1**, 48-52

Cao X (2002) Scientific bases of acupuncture analgesia. Acupunct Electrother Res **27:1,** 1-14

Cao XD (1997) From acupuncture anesthesia to acupuncture balance anesthesia, World J Acupunct Moxib **7**, 19–24

Carlsson C (2002) Acupuncture mechanisms for clinically relevant long-term effects--reconsideration and a hypothesis. Acupunct Med **20:2-3,** 82-99

Carlsson CP, Sundler F, Wallengren J (2006) Cutaneous innervation before and after one treatment period of acupuncture. Br J Dermatol **155:5,** 970-976

Carlton SM, Coggeshall RE (1997) Immunohistochemical localization of 5-HT2A receptors in peripheral sensory axons in rat glabrous skin. Brain Res **763,** 271–275

Carpenter JL, Andrews LK, Holzworth J: Tumors and tumor-like lesions. In: Holzworth J (ed.) Diseases of the cat: Medicine and surgery. Philadelphia: WB Saunders Company, 1987, pp. 406-596

Carr KD (1984) The physiology of opiate hedonic effects and the role of opioids in motivated behavior. Adv Alcohol Subst Abuse **3:3**, 5-18

Cassileth BR, Deng G (2004) Complementary and Alternative Therapies for Cancer. The Oncologist **9**, 80-89

Cassileth DR, Deng GE, Gomez JE, et al. (2007) Complementary therapies and integrative oncology in lung cancer: ACCP evidence-based clinical practice guidelines. Chest **132:3 (Suppl.),** 340S-354S

Catterall W (2000) From Ionic Currents to Molecular Mechanisms-The Structure and Function of Voltage-Gated Sodium Channels. Neuron **26:1**, 13-25

Ceccherelli F, Gagliardi G, Matterazzo G, et al. (1996) The role of manual acupuncture and morphine administration on the modulation of capsaicin-induced edema in rat paw. A blind controlled study. Acupunct Electrother Res **21:1**, 7-14

Chae Y, Park HJ, Hahm DH, et al. (2006) Individual differences of acupuncture analgesia in humans using cDNA microarray. J Physiol Sci **56**, 425–431

Chan CWY, Tsang H (1987) Inhibition of human flexion reflex by low intensity high frequency transcutaneous electrical nerve stimulation (TENS) has a gradual onset and offset. Pain **28**, 239–253

Chan WW, Chen KY, Liu H et al. (2001) Acupuncture for General Veterinary Practice. J Vet Med Sci **63:10,** 1057-1062

Chandrasekharan N, Dai H, Roos K, et al. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proc Natl Acad Sci USA **99:21**, 13926-13931

Chang FC, Tsai HY, Yu MC, et al. (2004) The Central Serotonergic System Mediates the Analgesic Effect of Electroacupuncture on Zusanli (ST36) Acupoints. J Biomed Sci **11,** 179-185

Chang HT (1973) Integrative action of thalamus in the process of acupuncture for analgesia. Sci Sin **16**, 25–60

Chang HT (1980) Neurophysiological interpretation of acupuncture analgesia. Endeavour **3**, 92–96

Chen XH, Geller EB, Adler MW (1998) CCK(B) receptors in the periaqueductal grey are involved in electroacupuncture antinociception in the rat cold water tail-flick test, Neuropharmacology **37**, 751–757

Cheng B, Liu HW, Fu XB, et al. (2008) Coexistence and upregulation of three types of opioid receptors, mu, delta and kappa, in human hypertrophic scars. Br J Dermatol **158:4**, 713-720

Cheng RS, Pomeranz B (1981) Monoaminergic mechanism of electroacupuncture analgesia. Brain Res **215:1-2**, 77-92

Chiang CY, Chang CT, Chu HC, et al. (1973) Peripheral afferent pathway for acupuncture analgesia. Sci Sin (B) **16**, 210–217

Chiang CY, Liu JY, Chu TH, et al. (1975) Studies of spinal ascending pathway for acupuncture analgesia. Sci Sin (B) **18,** 651–658

Chiu JH, Chung MS, Cheng HC, et al. (2003) Different central manifestations in response to electroacupuncture at analgesic and nonanalgesic acupoints

in rats: a manganese-enhanced functional magnetic resonance imaging study. Can J Vet Res **67:2**, 94-101

Cho ZH, Chung SC, Jones JP, et al. (1998) New findings of the correlation between acupoints and corresponding brain cortices using functional MRI. Proc Natl Acad Sci USA **95:5**, 2670-2673

Choi BT, Kang J, Jo UB (2005b) Effects of electroacupuncture with different frequencies on spinal ionotropic glutamate receptor expression in complete Freund's adjuvant-injected rat. Acta Histochem **107**, 67–76

Choi BT, Lee JH, Wan Y, et al. (2005a) Involvement of ionotropic glutamate receptors in low frequency electroacupuncture analgesia in rats. Neurosci Lett **377**, 185–188

Chun R, de Lorimier LP (2003) Update on the biology and management of canine osteosarcoma. Vet Clin North Am Small Anim Pract **33**, 491-516

Chung A, Bui L, Mills E (2003) Adverse effects of acupuncture. Which are clinically significant? Can Fam Physician **49**, 985-959

Chung JM, Fang ZR, Hori Y, et al. (1984) Prolonged inhibition of primate spinothalamic tract cells by peripheral nerve stimulation. Pain **19**, 259–275

Chung JM: Neurophysiological mechanisms of acupuncture analgesia in experimental animal models. In: Pomeranz B, Stus G (eds.) Scientific Bases of Acupuncture. Berlin: Springer-Verlag, 1989, pp. 137–155

Cleeland C, Gonin R, Hatfield A, et al. (1994) Pain and its treatment in outpatients with metastatic cancer. N Engl J Med **330:9**, 592-596

Cleton-Jansen AM, Anninga JK, Briaire-de Bruijn IH, et al. (2009) Profiling of high-grade central osteosarcoma and its putative progenitor cells identifies tumourigenic pathways. Br J Cancer **101:11**, 1909-18

Codman EA (1924) The Registry of Bone Sarcomas as an Example of the End-Result Idea in Hospital Organization. Bull Am Coll Surg **8**, 34–38

Codman EA (1926) Registry of Bone Sarcoma: Part I.—Twenty-Five Criteria for Establishing the Diagnosis of Osteogenic Sarcoma. Part II.—Thirteen Registered Cases of ‘‘Five Year Cures’’ Analyzed According to These Criteria. Surg Gynecol Obstet **42**, 381–393

Coggeshall RE (2005) Fos, nociception and the dorsal horn. Prog Neurobiol **77:5**, 299-352

Coggeshall RE, Carlton SM (1998) Ultrastructural analysis of NMDA, AMPA and kainite receptors on unmyelinated and myelinated axons in the periphery. J Comp Neurol **391**, 78–86

Colvin L, Fallon M (2008) Challenges in cancer pain management: bone pain. Eur J Cancer **44**, 1083–1090

Cook-Mozaffari P (1996) Cancer and fluoridation. Community Dent Health **13:Suppl. 2,** 56-62

Cooley DM, Waters DJ (1995) Skeletal neoplasms of small dogs. Proceedings, Veterinary Cancer Society, 22-23

Cooperman AM, Hall B, Mikalacki K, et al. (1977) Use of transcutaneous electrical stimulation in the control of postoperative pain: results of a prospective, randomized, controlled study. Amer J Surg **133**, 185-187

Cox BM, Opheim KE, Teschemacher H, et al. (1975) A peptide-like substance from pituitary that acts like morphine. 2. Purification and properties. Life Sci **16**, 1777-1782

Craft RM, Mogil JS, Aloisi AM (2004) Sex differences in pain and analgesia: the role of gonadal hormones. Euro J Pain **8,** 397-411

Crane SW (1987) Perioperative analgesia: A surgeon's perspective. JAVMA **191**, 1254-1257

Cui KM, Li WM, Gao X, et al. (2005) Electro-acupuncture relieves chronic visceral hyperalgesia in rats. Neurosci Lett **376**, 20–23

Dahlin DC, Unni KK: Bone Tumors: General Aspects and Data on 8542 Cases. 4th ed. Springfield, IL: Charles C. Thomas, 1986

Dai P, Wang ZJ, Sun WW, et al. (2009) Effects of electro-acupuncture on IGF-I expression in spared dorsal root ganglia and associated spinal dorsal horn in cats subjected to adjacent dorsal root ganglionectomies. Neurochem Res **34:11**, 1993-1998

Dai Y, Kondo E, Fukuoka T, et al. (2001) The effect of electroacupuncture on pain behaviors and noxious stimulus-evoked Fos expression in a rat model of neuropathic pain. J Pain **2**, 151–159

Daino K, Ugolin N, Altmeyer-Morel S, et al. (2009) Gene expression profiling of alpha-radiation-induced rat osteosarcomas: identification of dysregulated genes involved in radiation-induced tumorigenesis of bone. Int J Cancer **125:3**, 612-620

Dass CR, Choong PF (2007) GFP expression alters osteosarcoma cell biology. DNA Cell Biol **26:8**, 599-601

De Kock MF, Lavand'homme PM (2007) The clinical role of NMDA receptor antagonists for the treatment of postoperative pain. Best Pract Res Clin Anaesthesiol **21:1**, 85-98

de Medeiros MA, Canteras NS, Suchecki D, et al. (2003) Analgesia and c-Fos expression in the periaqueductal gray induced by electroacupuncture at the Zusanli point in rats. Brain Res **973**, 196–204

Delaney A, Fleetwood-Walker SM, Colvin LA, et al. (2008) Translational medicine: cancer pain mechanisms and management. Br J Anaesth **101**, 87–94

DeLeo JA (2006) Basic Science of Pain. J Bone & Joint Surg **88-A**:**Suppl. 2,** 58-62

DeLeo JA, Tanga FY, Tawfik VL (2004) Neuroimmune activation and neuroinflammation in chronic pain and opioid tolerance/hyperalgesia. Neuroscientist **10**, 40–52

Descartes R: Les passions de l'âme, Amsterdam, 1649 (In French.) Reprinted in AT, vol. XI. Electronic text available online. English translation in CSM, vol. I. Hackett English edition, trans. Stephen H. Voss, 1989

Descartes R: L'Homme, Paris, 1664 (In French.) Reprinted in Adam and Tannery vol. XI. Partial English translation in CSM, vol. I. Complete English translation in Hall TS: Treatise of Man, Harvard, Mass, 1972

Devor M, Govrin-Lippmann R, Angelides K (1993) Na+ channel immunolocalization in peripheral mammalian axons and changes following nerve injury and neuroma formation. J Neurosci **13**, 1976–1992

Dickens DS, Kozielski R, Khan J, et al. (2002) Cyclooxygenase-2 expression in pediatric sarcomas. Pediatr Dev Pathol **5**, 356-64

Dimopoulou M, Kirpensteijn J, Moens H, et al. (2008) Histologic prognosticators in feline osteosarcoma: a comparison with phenotypically similar canine osteosarcoma. Vet Surg **37:5**, 466-471

Dina O, Chen X, Reichling D, et al. (2001) Role of protein kinase Cepsilon and protein kinase A in a model of paclitaxel-induced painful peripheral neuropathy in the rat. Neuroscience **108:3**, 507-515

Dong WQ, He LF, Wang MZ, et al. (1987) Effects of iontophoretic etrophine and electroacupuncture on evoked neuronal response in anterior nuclei of rabbits' thalamus. Chin J Physiol Sci **3**, 244–250

Dong XW, Jiang ZH, Fu LW (1978) Changes in monoamine fluorescence intensity in the rat's midbrain raphe nuclei and locus coeruleus in the process of acupuncture analgesia. [Article in Chinese, English abstract]. Acta Biochem Biophys Sin **10**, 119–125

Dong ZQ, Xie H, Ma F, et al. (2005) Effects of electroacupuncture on expression of somatostatin and preprosomatostatin mRNA in dorsal root ganglions and spinal dorsal horn in neuropathic pain rats. Neurosci Lett **385**, 189–194

Donovan KA, Taliaferro LA, Brock CW, et al. (2008) Sex Differences in the Adequacy of Pain Management Among Patients Referred to a Multidisciplinary Cancer Pain Clinic. J Pain Sympt Manag **36:2**, 167-172

Dougherty PM, Willis WD (1991) Enhancement of spinothalamic neuron responses to chemical and mechanical stimuli following combined micro-iontophoretic application of N-methyl-d-aspartic acid and substance P. Pain **47,** 85–93

Du HJ, Zhao YF (1975) Central localization of descending inhibition of visceral-somatic reflex produced by acupuncture. Sci Sin (B) **18**, 631–639

Du HJ, Zhao YF (1976) Localization of central structures involved in descending inhibitory effect of acupuncture on visceral-somatic reflex discharges. Sci Sin (B) **19**, 137–148

Du J, He L (1992) Alterations of spinal dorsal horn substance P following electroacupuncture analgesia—a study of the formalin test with immunohistochemistry and densitometry. Acupunct Electrother Res **17**, 1–6

Du J, Zhou S, Coggeshall RE, et al. (2003) N-Methyl-d-aspartate-induced excitation and sensitization of normal and inflamed nociceptors. Neuroscience **118**, 547–562

Dualé C, Sibaud F, Guastella V, et al. (2009) Perioperative ketamine does not prevent chronic pain after thoracotomy. Eur J Pain **13:5**, 497-505

Dubielzig RR, Biery DN, Brodey RS (1981) Bone sarcomas associated with multifocal medullary bone infarction in dogs. JAVMA **179**, 64-68

Duggan AW, Hendry IA, Morton CR, et al. (1987) Cutaneous stimuli releasing immunoreactive substance P in the dorsal horn of the cat. Brain Res **451**, 261–273

Edwards JR, Williams K, Kindblom LG, et al. (2008) Lymphatics and bone. Hum Pathol **39:1**, 49-55

Ee CC, Manheimer E, Pirotta MV, et al. (2008) Acupuncture for pelvic and back pain in pregnancy: a systematic review. Am J Obstet Gynecol **198:3**, 254-259

Ehernpreis S, Balagot C, Comathy J, et al. (1978) Narcotic reversible analgesia in mice produced by d-phenylanaline and hydrocinnamic acid, inhibitors of carboxypeptidase A. Adv Pain Res Ther **3**, 479–488

Eisenberg E, Pud D, Koltun L, et al. (2007) Effect of early administration of the N-methyl-d-aspartate receptor antagonist amantadine on the development of postmastectomy pain syndrome: a prospective pilot study. J Pain **8:3**, 223-229

Elliott AA, Elliott JR (1993) Tetrodotoxin-resistant Na+ currents and inflammatory hyperalgesia, J Physiol **463**, 39–56

Elliott TE, Elliott BA (1992) Physician attitudes and beliefs about use of morphine for cancer pain. J Pain Symptom Manag **7:3**, 141-148

Ellison NM, Chevlen EM: Palliative Chemotherapy. In: Berger AM, Portenoy RK, Weissman DE (eds.) Principles and Practice of Palliative Care and Supportive Oncology. Philadelphia: Lippincott Williams & Wilkins, 2002, pp. 698-709

Enneking WF, Spanier SS, Goodman MA (1980a) Current concepts review. The surgical staging of musculoskeletal sarcoma. J Bone Joint Surg Am **62:6**, 1027-1030

Enneking WF, Spanier SS, Goodman MA (1980b) A system for the surgical staging of musculoskeletal sarcoma. Clin Orthop Relat Res **153**, 106-120

Epstein JB, Wilkie DJ, Fischer DJ, et al. (2009) Neuropathic and nociceptive pain in head and neck cancer patients receiving radiation therapy. Head Neck Oncol **1:1**, 26

Ericson M, Gabrielson A, Worel S, et al. (1999) Substance P (SP) in innervated and non-innervated blood vessels in the skin of patients with symptomatic scalp. Exp Dermatol **8:4**, 344-345

Ernst M, Lee MH (1987) Influence of naloxone on electro-acupuncture analgesia using an experimental dental pain test. Review of possible mechanisms of action. Acupunct Electrother Res **12:1**, 5-22

Eyre R, Feltbower RG, Mubwandarikwa E, et al. (2009) Epidemiology of bone tumours in children and young adults. Pediatr Blood Cancer **53:6**, 941-952

Ezzo J, Berman B, Hadhazy VA, et al. (2000) Is acupuncture effective for the treatment of chronic pain? A systematic review. Pain **86**, 217–225

Fan SG, Qu ZC, Zhe QZ, et al. (1982) GABA: antagonistic effect on electroacupuncture analgesia and morphine analgesia in the rat. Life Sci **31:12-13**, 1225-1228

Fan TM, Lorimier LP, Charney SC et al. (2007) Single-Agent Pamidronate for Palliative Therapy of Canine Appendicular Osteosarcoma Bone Pain. J Vet Intern Med **21**, 431-439

Fang JL, Krings T, Weidemann J, et al. (2004) Functional MRI in healthy subjects during acupuncture: different effects of needle rotation in real and sham acupoints. Neuroradiology **46,** 359–362

Fang Z, Yu Q, Li Y (1993) The correlation between postsynaptic inhibition and GABA, opioid peptides, SP in electroacupuncture. [Article in Chinese, English abstract]. Zhen Ci Yan Jiu **18:1**, 64-67

Farber PL, Tachibana A, Campiglia HM (1997) Increased pain threshold following electroacupuncture: analgesia is induced mainly in meridian acupuncture points. Acupunct Electrother Res **22**, 109–117

Faris PL, Komisaruk BR, Watkin LR (1983) Evidence for the neuropeptide cholecystokinin as an antagonist of opiate analgesia. Science **219**, 310–312

Fei H, Xie GX, Han JS (1987) Low and high frequency electroacupuncture stimulation release [Met5]enkephalin and dynorphin A in rat spinal cord. Sci Bull China **32,** 1496–1501

Fields HL, Basbaum AI, Heinricher MM: Central nervous system mechanisms of pain modulation. In: Wall P, Melzack R (eds.) Text book of Pain (5th ed.). Elsevier, 2005

Fillingim RB, Ness TJ (2000) Sex-related hormonal influences on pain and analgesic responses. Neurosci Biobehav Rev **24,** 485-501

Finkelstein MM, Kreiger N (1996) Radium in drinking water and risk of bone cancer inOntario youths: a second study and combined analysis. Occup Environ Med **53:5**, 305-311

Fitzgerald M, Woolf CJ: Axon transport and sensory C fibre function. In: Chahl LA, Szolcsanyi J, Lembeck F (eds) Antidromic Vasodilation and Neurogenic Inflammation. Budapest: Akademiai Kiado, 1984, pp.119-140

Fohr B, Schulz A, Battmann A (2000) Sex steroids and bone metabolism: comparison of in vitro effects of 17beta-estradiol and testosterone on human osteosarcoma cell lines of various gender and differentiation. Exp Clin Endocrinol Diabetes **108**, 414-423

Folio C, Mora MI, Zalacain M, et al. (2009) Proteomic analysis of chemonaive pediatric osteosarcomas and corresponding normal bone reveals multiple altered molecular targets. J Proteome Res **8:8**, 3882-3888

Folkman J (1995) Seminars in Medicine of the Beth Israel Hospital, Boston. Clinical applications of research on angiogenesis. N Engl J Med **333:26**, 1757-1763

Fraumeni JF (1967) Stature and malignant tumors of bone in childhood and adolescence. Cancer **20**, 967-973

Freedman RR (2005) Pathophysiology and treatment of menopausal hot flashes. Seminars in Reproductive Medicine **23:2,** 117-25

Fuchs N, Bielack SS, Epler D, et al. (1998) Long-term results of the co-operative German-Austrian-Swiss osteosarcoma study group's protocol COSS-86 of intensive multidrug chemotherapy and surgery for osteosarcoma of the limbs. Ann Oncol **9:8**, 893-899

Fuhrhop I, Schroeder M, Rafnsdóttir SL, et al. (2009) Dynamics of microvascular remodelling during tumor growth in bone. J Orthop Res **28:1**, 27-31

Fung SJ, Chan SHH (1976) Primary afferent depolarization evoked by electroacupuncture in the lumbar cord of the cat. Exp Neurol **52**, 168–176

Fusumada K, Yokoyama T, Miki T, et al. (2007) c-Fos expression in the periaqueductal gray is induced by electroacupuncture in the rat, with possible reference to GABAergic neurons. Okajimas Folia Anat Jpn **84,** 1–9

Gach K, Piestrzeniewicz M, Fichna J, et al. (2009) Opioid antagonist-induced regulation of the mu-opioid receptor expression in MCF-7 breast cancer cell line. Endocr Regul **43:1**, 23-28

Gammaitoni A, Davis M (2002) Pharmacokinetics and tolerability of lidocaine patch 5% with extended dosing. Ann Pharmacother **36:2**, 236-240

Gao YJ, Ren WH, Zhang TQ, et al. (2004) Contributions of the anterior cingulate cortex and amygdala to pain- and fear- conditioned place avoidance in rats. Pain **110**, 343–353

Gareus IK, Lacour M, Schulte AC, et al. (2002) Is there a BOLD response of the visual cortex on stimulation of the vision-related acupoint GB 37? J Magn Reson Imaging **15:3**, 227-232

Garzotto CK, Berg J, Hoffmann WE, et al. (2000) Prognostic significance of serum alkaline phosphatase activity in canine appendicular osteosarcoma. J Vet Intern Med **14**, 587-592

Gaynor JS (2008) Control of cancer pain in veterinary patients. Vet Clin North Am Small Anim Pract **38:6**, 1429-48

Geber C, Baumgärtner U, Schwab R (2009) Revised definition of neuropathic pain and its grading system: an open case series illustrating its use in clinical practice. Am J Med **122:10 Suppl.,** S3-12

Gelberg KH, Fitzgerald EF, Hwang SA, et al. (1995) Fluoride exposure and childhood osteosarcoma: a case-control study. Am J Pub Health **85**, 1678-1683

Gelberg KH, Fitzgerald EF, Hwang SA, et al. (1997) Growth and development and other risk factors for osteosarcoma in children and adults. In J Epidemiol **26**, 272-278

Gillette SM, Gillette EL, Powers BE, et al. (1990) Radiation-induced osteosarcoma in dogs after external beam or intraoperative radiation therapy. Cancer Res **50:1**, 54-57

Gleditsch J (2001) 50 Jahre DÄGfA. Zur Geschichte der deutschen Ärztegesellschaft für Akupunktur. [Article in German] Deutsche Zeitschrift für Akupunktur **Sondernummer 2a,** 176-191

Goblirsch MJ, Zwolak P, Clohisy DR (2005) Advances in understanding bone cancer pain. J Cell Biochem **96**, 682–688

Goldstein A, Tachibana S, Lowney LI, et al. (1979) Dynorphin-(1-13), an extraordinarily potent opioid peptide. Proc Natl Acad Sci **76**, 6666-6670

Goldstein A: The dynorphin (kappa opioid) receptor. In: Illes P, Farsang C (eds.) Regulatory Roles of Opioid Peptides. Weinheim: VCH Verlag, 1988

Goodman AJ, Le Bourdonnec B, Dolle RE (2007) Mu opioid receptor antagonists: recent developments. Chem Med Chem **2:11**, 1552-1570

Goodman RR, Snyder SH, Kuhar MJ, et al. (1980). Differentiation of delta and mu opiate receptor localizations by light microscopic autoradiography. Proc Natl Acad Sci **77**, 6239-6243

Gordon-Williams RM, Dickenson AH (2007) Central neuronal mechanisms in cancer-induced bone pain. Curr Opin Support Palliat Care **1:1,** 6-10

Gori L, Firenzuoli F (2007) Ear Acupuncture in European Traditional Medicine. eCAM **4:S1**, 13–16

Grond S, Radbruch L, Meuser T, et al. (1999) High-dose tramadol in comparison to low-dose morphine for cancer pain relief. J Pain Symptom Manage **18:3**, 174-179

Guan X, Shi J, Zhang Y, et al. (1990) The change of ACh metabolism in the dorsal horn of spinal cord and spinal ganglia during electroacupuncture analgesia (EAA). [Article in Chinese, English abstract]. Zhen Ci Yan Jiu **15:1**, 18-23

Guan XM, Wang CY, Liu XC, et al. (1984) The influence of ACh on the metabolism of monoamine transmitters in the brain during acupuncture analgesia. The Second National Symposium on Acupuncture and Moxibustion and Acupuncture Anesthesia Beijing, All-China Society of Acupuncture and Moxibustion, 409–410

Gunn C (1978) Motor points and motor lines. Am J Acupunct **5**, 55-58

Gunthorpe MJ, Szallasi A (2008) Peripheral TRPV1 receptors as targets for drug development: new molecules and mechanisms. Curr Pharm Des **14:1**, 32–41

Guo HF, Tian JH, Wang XM, et al. (1996) Brain substrates activated by electroacupuncture EA of different frequencies II: role of Fos/Jun proteins in EA-induced transcription of preproenkephalin and preprodynorphin genes. Mol Brain Res **43**, 167–173

Guo Z (1995) Chinese Confucian Culture and the Medical Ethical Tradition. J Med Ethics **21:4**, 239-246

Guo ZL, Moazzamia AR, Longhursta JC (2004) Electroacupuncture induces c-Fos expression in the rostral ventrolateral medulla and periaqueductal gray in cats: relation to opioid containing neurons. Brain Res **1030,** 103–115

Ha H, Tan EC, Fukunaga H, et al. (1991) Naloxone reversal of acupuncture analgesia in the monkey. Exp Neurol **73**, 298–303

Haake M, Müller HH, Schade-Brittinger C, et al. (2007) German Acupuncture Trials (GERAC) for Chronic Low Back Pain. Arch Intern Med **167:17,** 1892-1898

Haigler HJ (1978) Morphine: effects on serotonergic neurons and neurons in areas with a serotonergic input. Eur J Pharmacol **51:4**, 361-376

Haker E, Egekvist H, Bjerring P (2000). Effect of sensory stimulation (acupuncture) on sympathetic and parasympathetic activities in healthy subjects. J Auton Nerv Syst **79:1**, 52-59

Haker E, Lundeberg T (1990) Acupuncture treatment in epicondylalgia: a comparative study of two acupuncture techniques. Clin J Pain **6**, 221–226

Hald A, Nedergaard S, Hansen RR, et al. (2009) Differential activation of spinal cord glial cells in murine models of neuropathic and cancer pain. Eur J Pain **13:2,** 138-145

Haltrecht H. Veterinary acupuncture. Can Vet J **40:6**, 401-403

Hamilton GR, Baskett TF (2000) In the arms of Morpheus: the development of morphine for postoperative pain relief. Can J Anesth **47**, 367-374

Hamon M, Bourgoin S: Serotonin and its receptors in pain control. In: Sawynok J, Cowan A (eds.) Novel Aspects of Pain Management: Opioids and Beyond. New York: John Wiley & Sons Inc., 1999, pp. 203–228

Hamon M, Gallissot MC, Menard F, et al. (1989) 5-HT3 binding sites are on capsaicin-sensitive fibers in the rat spinal cord. Eur J Pharmacol **164**, 315–322

Han C, Li X, Luo H, et al. (2004) Clinical study on electro-acupuncture treatment for 30 cases of mental depression. J Tradit Chin Med **24:3**, 172-176

Han JS (1986) Electroacupuncture: An alternative to antidepressants for treating affective diseases? J Neurosci **29**, 79–92

Han JS (1995a) The future of acupuncture anesthesia, from acupuncture anesthesia (AA) to acupuncture-assisted anesthesia (AAA). Chin J Pain Med **2**, 1-5

Han JS (1995b) Cholecystokinin octapeptide (CCK-8): a negative feedback control mechanism for opioid analgesia. Prog Brain Res **105**, 263–271

Han JS (1997) Acupuncture anesthesia versus acupuncture-assisted anesthesia. World J Acupunct Moxib **7**, 16–18

Han JS (2003) Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. Trends Neurosci **26**, 17–22

Han JS, Chen XH, Sun SL, et al. (1991) Effect of low- and high-frequency TENS on met-enkephalin-arg-phe and dynorphin A immunoreactivity in human lumbar CSF. Pain **47**, 295-298

Han JS, Chou PH, Lu CH, et al. (1979a) The role of central 5-HT in acupuncture analgesia. Sci Sin **22**, 91–104

Han JS, Ren MF, Tang J, et al. (1979b) The role of catecholamine in acupuncture analgesia. Chin Med J **92,** 793–800

Han JS, Tang J, Ren MF, et al. (1980) Central neurotransmitters and acupuncture analgesia. Am J Chin Med **8**, 331–348

Han JS, Terenius L (1982) Neurochemical basis of acupuncture analgesia. Ann Rev Pharmacol Toxicol **22**, 193–220

Han JS, Wii LZ, Cui CL (1994) Heroin addicts treated with transcutaneous electrical nerve stimulation of identified frequencies. Regul Pept **54**, 115-116

Han JS, Xie GX, Zhou ZF (1984) Acupuncture mechanisms in rabbits studied with microinjection of antibodies against β-endophin, enkephalin and substance P. Neuropharmacology **23**, 1–5

Han JS, Xie GX, Zou D, et al. (1982) Enkephalin and B-endorphin as mediators of electro-acupuncture analgesia in rabbits, an antiserum microinjection study. Adv Biochem Psychopharmacol **33**, 368-377

Han JS, Zhang YZ, Zhou ZF, et al. (1981) Augmentation of acupuncture analgesia by peptidase inhibitor d-phenylalanine in rabbits. [Article in Chinese, English abstract]. Acta Zool Sin **27**, 133–137

Han JS, Zhou Z, Xuan Y (1983) Acupuncture has an analgesic effect in rabbits. Pain **15**, 83–91

Han JS: Central neurotransmitters and acupuncture analgesia. In: Pomeranz B, Stus G (eds.) Scientific Bases of Acupuncture. Berlin: Springer-Verlag, 1989, pp. 7–33

Han N, Luo F, Bian Z, et al. (2000) Synergistic effect of cholecystokinin octapeptide and angiotensin II in reversal of morphine induced analgesia in rats. Pain **85**, 465–469

Hang TT, CR Dass, PFM Choong, et al. (2009) Osteosarcoma treatment: state of the art. Cancer Metastasis Rev **28**, 247–263

Hansen MF, Koufos A, Gallie BL, et al. (1985) Osteosarcoma and retinoblastoma: a shared chromosomal mechanism revealing recessive predisposition. Proc Natl Acad Sci **82**, 6216-6220

Hansen MF, Seton M, Merchant A (2006) Osteosarcoma in Paget's disease of bone. J Bone Miner Res **21:Suppl 2.,** P58-63

Hansen-Algenstaedt N, Joscheck C, Schaefer C, et al. (2005) Long-term observation reveals time-course-dependent characteristics of tumour vascularisation. Eur J Cancer **41:7**, 1073-1085

Harris JA (1998) Using c-fos as a neural marker of pain. Brain Res Bull **45:1**, 1-8

Harris MB, Gieser P, Goorin AM, et al. (1998) Treatment of metastatic osteosarcoma at diagnosis: a Pediatric Oncology Group Study. J Clin Oncol **16:11**, 3641-3648

Harris RE (ed.) COX-2 Blockade in Cancer Prevention and Therapy. Totowa, NJ: Humana Press, 2003

Harris RE, Zubieta JK, Scott DJ, et al. (2009) Traditional Chinese acupuncture and placebo (sham) acupuncture are differentiated by their effects on mu-opioid receptors (MORs). Neuroimage **47:3**, 1077-1085

Hawkins MM, Wilson LMK, Burton HS, et al. (1996) Radiotherapy, alkylating agents, and risk of bone cancer after childhood cancer. J Natl Cancer Inst **88**, 270-278

Hayashi AM, Matera JM, Fonseca Pinto AC (2007) Evaluation of electroacupuncture treatment for thoracolumbar intervertebral disk disease in dogs. J Am Vet Med Assoc **231:6**, 913-918

He LF (1987) Involvement of endogenous opioid peptides in acupuncture analgesia. Pain **31**, 99–121

He LF, Lu RL, Zhuang SY (1985) Possible involvement of opioid peptides of caudate nucleus in acupuncture analgesia. Pain **23**, 83–93

He LF, Xu SF (1981) Caudate nucleus and acupuncture analgesia. Acupunct Electrother Res Int J **6**, 169–183

Heldmann E, Anderson MA, Wagner-Mann C (2000) Feline osteosarcoma: 145 cases (1990–1995). J Am Anim Hosp Assoc **36**, 518–521

Hellyer P, Rodan I, Brunt J, et al. (2007) AAHA/AAFP pain management guidelines for dogs & cats. J Am Anim Hosp Assoc **43:5**, 235-48

Hendrich J, Van Minh AT, Heblich F, et al. (2008) Pharmacological disruption of calcium channel trafficking by the alpha2delta ligand gabapentin. Proc Natl Acad Sci USA **105:9**, 3628–33

Herrero JF, Laird JM, López-García JA (2000) Wind-up of spinal cord neurones and pain sensation: much ado about something? Prog Neurobiol **61:2**, 169-203

Hillner B, Ingle J, Berenson J, et al. (2000) American Society of Clinical Oncology guideline on the role of bisphosphonates in breast cancer. American Society of Clinical Oncology Bisphosphonates Expert Panel. J Clin Oncol **18:6**, 1378-1391

Hirshberg RM, Al-Chaer ED, Lawand NB, et al. (1996) Is there a pathway in the posterior funiculus that signals visceral pain? Pain **67**, 291-305

Hjorth S (1993) Serotonin 5-HT1A autoreceptor blockade potentiates the ability of the 5-HT reuptake inhibitor citalopram to increase nerve terminal output of 5-HT in vivo; a microdialysis study. J Neurochem **60,** 776–779

Hoheisel U, Koch K, Mense S (1994) Functional reorganization in the rat dorsal horn during an experimental myositis. Pain **59:1**, 111-118

Hökfelt T, Ljungdahl A, Terenius L, et al. (1977) Immunohistochemical analysis of peptide pathways possibly related to pain and analgesia: enkephalin and substance P. Proc Natl Acad Sci USA **74:7**, 3081-3085

Hökfelt T, Vincent SR, Dalsgaard CJ, et al. (1984) Some aspects on distribution and role of opioid peptides in the central and peripheral nervous system. In: Müller EE, Genazzani AR (eds.) Central and Peripheral Endorphins: Basic and Clinical Aspects. New York: Raven, 1984

Holdsworth M, Forman W, Killilea T, et al. (1994) Transdermal fentanyl disposition in elderly subjects. Gerontology **40:1**, 32-37

Hortobagyi GN, Theriault RL, Porter L, et al. (1996) Efficacy of pamidronate in reducing skeletal complications in patients with breast cancer and lytic bone metastases. Protocol 19 Aredia Breast Cancer Study Group. N Engl J Med **335:24**, 1785-1791

Hoshino S, Inoue S, Hosoi T, et al. (1995) Demonstration of isoforms of the estrogen receptor in the bone tissues and in osteoblastic cells. Calcif Tissue Int **57:6**, 466-468

Hsieh JC, Tu CH, Chen FP, et al. (2001) Activation of the hypothalamus characterizes the acupuncture stimulation at the analgesic point in human: a positron emission tomography study. Neurosci Lett **307,** 105–108

Hu JY, Zhao ZQ (2001) Differential contributions of NMDA and non-NMDA receptor to spinal c-Fos expression evoked by superficial tissue and muscle inflammation in the rat. Neuroscience **106**, 823–831

Hu SJ, Hu JJ, Fan JZ (1980) The influence of dorsal half transection of the spinal cord on inhibitory effect of electroacupuncture upon the medbrain discharges. [Article in Chinese, English abstract]. Acta Zool Sin **26**, 115–120

Hu ZL (1979) A study on the histological structure of acupuncture points and types of fibers conveying needling sensation. Chin Med J **92**, 233

Huang C, Hua ZP, Jiang SZ, et al. (2007) CCK-8 receptor antagonist L365,260 potentiates the efficacy to and reverses chronic tolerance to electroacupuncture-induced analgesia in mice. Brain Res Bull **71**, 447–451

Huang C, Li HT, Shi YS, et al. (2004) Ketamine potentiates the effect of electroacupuncture on mechanical allodynia in a rat model of neuropathic pain. Neurosci Lett **368,** 327–331

Huang C, Wang Y, Han JS, et al. (2002) Characteristics of electroacupuncture-induced analgesia in mice: variation with strain, frequency, intensity and opioid involvement. Brain Research **945,** 20-25

Hughes J (1975) Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. Brain Res **88**, 295-308

Hughes J, Smith TW, Kosterlitz HW, et al. (1975) Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature **258**, 577-579

Hui KK, Liu J, Makris N, et al. (2000) Acupuncture modulates the limbic system and subcortical gray structures of the human brain: evidence from fMRI studies in normal subjects. Hum Brain Ma **9**, 13–25

Hui KK, Liu J, Marina O, et al. (2005) The integrated response of the human cerebro-cerebellar and limbic systems to acupuncture stimulation at ST 36 as evidenced by fMRI. Neuroimage **27**, 479–496

Hunt SP, Mantyh PW (2001) The molecular dynamics of pain control. Nat Rev Neurosci **2**, 83–91

Ikeda T, Shigeno C, Kasai R, et al. (1993) Ovariectomy decreases the mRNA levels of transforming growth factor-beta 1 and increases the mRNA levels of osteocalcin in rat bone in vivo. Biochem Biophys Res Commun **194:3**, 1228-1233

Im EO, Chee W (2001) A feminist critique of research on cancer pain. West J Nurs Res **23:7**, 726-752

Ito S, Katssura G, Maeda Y (1982) Caerulein and CCK suppress endorphin induced analgesia in the rat. Eur J Pharmacol **80**, 270–289

Iwa M, Strickland C, Nakade Y, et al. (2005) Electroacupuncture reduces rectal distension-induced blood pressure changes in conscious dogs. Dig Dis Sci **50:7**, 1264-1270

Jaffe HL: Tumors and Tumorous Conditions of the Bones and Joints. Lea & Febiger: Philadelphia, 1958

Jaffe N (1998) Historical perspective of the treatment of osteosarcoma: an interview with Dr Norman Jaffe. Interview by Margaret Pearson. J Pediatr Oncol Nurs **15:2**, 90-94

Janssens L, Rogers P, Schoen A (1988) Acupuncture Analgesia: A Review. Vet Rec **122**, 355-358

Jeffree GM, Price CH, Sissons HA (1975) The metastatic patterns of osteosarcoma. Br J Cancer **32:1**, 87-107

Jellinger KA (2009) Functional pathophysiology of consciousness. [Article in German] Neuropsychiatr **23:2**, 115-133

Jenkins C, Bruera E (1999) Nonsteroidal anti-inflammatory drugs as adjuvant analgesics in cancer patients. Palliat Med **13:3**, 183-196

Jenkins WL (1987) Pharmacologic aspects of analgesic drugs in animals: an overview. JAVMA **191**, 1231-1240

Jensen H, Zesler R, Christensen T (1991) Transcutaneous electrical nerve stimulation (TNS) for painful osteoarthrosis of the knee. Int J Rehabil Res **14:4**, 356-358

Jensen TS, Madsen CS, Finnerup NB (2009) Pharmacology and treatment of neuropathic pains. Curr Opin Neurol **22:5**, 467-474

Jensen TS: Mechanisms of neuropathic pain. In: Campbell JN (ed.) Pain - An Updated Review. Seattle: IASP Press, 1996

Ji GC, Yu J, Dong ZQ, et al. (2003) Changes of expression of IL-1 receptor I mRNA in rat periaqueductal gray after peripheral inflammation and electroacupuncture analgesia. Acupunct Res **28,** 111–140

Ji RR, Befort K, Brenner GJ, et al. (2002) ERK MAP kinase activation in superficial spinal cord neurons induces prodynorphin and NK-1 upregulation and contributes to persistent inflammatory pain hypersensitivity. J Neurosci **22,** 478–485

Ji RR, Zhang Q, Han JS (1993a) Electroacupuncture stimulation induced the synthesis of Fos-like protein in rat spinal cord. [Article in Chinese, English abstract]. Acta Physiol Sin **44**, 394–400

Ji RR, Zhang Q, Han JS (1993b) Electroacupuncture stimulation promotes preproenkephalin mRNA expression in rat spinal cord and medulla. [Article in Chinese, English abstract]. Acta Physiol Sin **45**, 395–399

Jiang ZY, Ye Q, Shen YT, et al. (1978) Effects of naloxone on experimental AA evaluated by sensory decision theory. Acta Zool Sin **24**, 1–10

Jimenez-Andrade JM, Mantyh WG, Bloom AP, et al. (2009) A phenotypically restricted set of primary afferent nerve fibers innervate the bone versus skin: Therapeutic opportunity for treating skeletal pain. Bone **Sep 18** [Epub ahead of print]

Jin H, Zhou L, Chang T, et al. (1992) The inhibition by electrical stimulation on gastric acid secretion is mediated by endorphin and somatostatin in dogs. Clin Res **40**, 167A

Johansson K, Lindgren I, Widner H, et al. (1993) Can sensory stimulation improve the functional outcome of stroke patients? Neurology **43**, 2189-2192

Jones CJ, Budsberg SC (2000) Physiologic characteristics and clinical importance of the cyclooxygenase isoforms in dogs and cats. JAVMA **217**, 721-727

Juarez G, Ferrell B, Borneman T (1998) Influence of culture on cancer pain management in Hispanic patients. Cancer Pract **6:5**, 262-269

Juarez G, Ferrell B, Borneman T (1999) Cultural considerations in education for cancer pain management. J Cancer Educ **14:3**, 168-173

Kang JM, Park HJ, Choi YG, et al. (2007) Acupuncture inhibits microglial activation and inflammatory events in the MPTP-induced mouse model. Brain Res **1131**, 211–219

Kaptchuk T (1997) Acupuncture: History, Context, and Long-Term Perspectives. NIH Consensus Development Conference on Acupuncture, November 3-5, Bethesda, Maryland

Kavoussi B, Ross BE. The Neuroimmune Basis of Anti-inflammatory Acupuncture. Integr Cancer Ther **6**, 251-257

Kawakita K, Funakoshi M (1982) Suppression of the jaw-opening reflex by conditioning a-delta fiber stimulation and electroacupuncture in the rat. Exp Neurol **78**, 461–465

Kawakita K, Itoh K, Okada K (2002) The polymodal receptor hypothesis of acupuncture and moxibustion, and its rational explanation of acupuncture points. Int Congress Ser **1238**, 63–68

Kay-Mugford P, Benn SJ, LaMarre J, et al. (2000) In vitro effects of nonsteroidal anti-inflammatory drugs on cyclooxygenase activity in dogs. AJVR **61**, 802-810

Kayser V, Elfassi IE, Aubel B, et al. (2007) Mechanical, thermal and formalin-induced nociception is differentially altered in 5-HT1A−/−, 5-HT1B−/−, 5-HT2A−/−, 5-HT3A−/− and 5-HTT−/− knock-out male mice. Pain **130**, 235–248

Kazmierski KJ, Ogilvie GK, Fettman MJ, et al. (2001) Serum zinc, chromium, and iron concentrations in dogs with lymphoma and osteosarcoma. J Vet Intern Med **15**, 585-588

Khanna C, Prehn J, Yeung C, et al. (2000) An orthotopic model of murine osteosarcoma with clonally related variants differing in pulmonary metastatic potential. Clin Exp Metastasis **18,** 261-271

Khasabov SG, Hamamoto DT, Harding-Rose C, et al. (2007) Tumor-evoked hyperalgesia and sensitization of nociceptive dorsal horn neurons in a murine model of cancer pain. Brain Research **1180,** 7-19

Kieffer BL (1995) Recent advances in molecular recognition and signal transduction of active peptides: receptors for opioid peptides. Cell Molec Neurobio **15**, 615-635

Kim HW, Roh DH, Yoon SY, et al. (2006) The Anti-Inflammatory Effects of Low- and High-Frequency Electroacupuncture Are Mediated by Peripheral Opioids in a Mouse Air Pouch Inflammation Model. J Altern Complement Med **12:1,** 39-44

Kim JH, Min BI, Na HS, et al. (2004) Relieving effects of electroacupuncture on mechanical allodynia in neuropathic pain model of inferior caudal trunk injury in rat: mediation by spinal opioid receptors. Brain Res **998**, 230–236

Kim JH, Min BI, Schmidt D, et al. (2000) The difference between electroacupuncture only and electroacupuncture with manipulation on analgesia in rats. Neurosci Lett **279**, 149–152

Kim MS, Lee SY, Cho WH, et al. (2008) Initial tumor size predicts histologic response and survival in localized osteosarcoma patients. J Surg Oncol **97:5**, 456-461

Kim SK, Park JH, Ba SJ, et al. (2005) Effects of electroacupuncture on cold allodynia in a rat model of neuropathic pain: mediation by spinal adrenergic and serotonergic receptors. Exp Neurol **195,** 430–436

Kimberlin C, Brushwood D, Allen W, et al. (2004) Cancer patient and caregiver experiences: communication and pain management issues. J Pain Symptom Manag **28:6**, 566-578

King T, Vardanyan A, Majuta L, et al. (2007) Morphine treatment accelerates sarcoma-induced bone pain, bone loss, and spontaneous fracture in a murine model of bone cancer. Pain **132:1-2,** 154-168

Kissin I (2008) Vanilloid-induced conduction analgesia: selective, dose-dependent, long-lasting, with a low level of potential neurotoxicity. Anesth Analg **107:1**, 271-281

Kitchell RL (1987) Problems in defining pain and peripheral mechanisms of pain. JAVMA **191**, 1195-1199

Klein MJ, Siegal GP (2006) Osteosarcoma. Anatomic and Histologic Variants. Am J Clin Pathol **125**, 555-581

Klenke FM, Gebhard MM, Ewerbeck V, et al. (2006) The selective Cox-2 inhibitor Celecoxib suppresses angiogenesis and growth of secondary bone tumors: an intravital microscopy study in mice. BMC Cancer **6**, 9

Ko ES, Kim SK, Kim JT, et al. (2006) The difference in mRNA expressions of hypothalamic CCK and CCK-A and -B receptors between responder and non-responder rats to high frequency electroacupuncture analgesia. Peptides **27**, 1841–1845

Ko J, Na DS, Lee YH, et al. (2002) cDNA microarray analysis of the differential gene expression in the neuropathic pain and electroacupuncture treatment models. J Biochem Mol Biol **35**, 420–427

Kong J, Fufa DT, Gerber AJ, et al. (2005) Psychophysical outcomes from a randomized pilot study of manual, electro, and sham acupuncture treatment on experimentally induced thermal pain. J Pain **6**, 55–64

Kong J, Gollub R, Huang T, et al. (2007) Acupuncture De Qi, from qualitative history to quantitative measurement. J Altern Complement Med **13:10**, 1059-1070

Kong J, L. Ma, R.L. Gollub, et al. (2002) A pilot study of functional magnetic resonance imaging of the brain during manual and electroacupuncture stimulation of acupuncture point (LI-4 Hegu) in normal subjects reveals differential brain activation between methods. J Altern Complement Med **8,** 411–419

Kong T, Fan T, Chu X (1991) Studies on the relationship between acupuncture analgesia and testosterone or dihydrotestosterone in blood plasma. [Article in Chinese, English abstract]. Zhen Ci Yan Jiu **16:2**, 138-141

Kosterlitz HW, Hughes J (1975) Some thoughts on the significance of enkephalin, the endogenous ligand. Life Sci **17**, 91-96

Kramarova E, Stiller CA (1996) The international classification of childhood cancer. Int J Cancer **68**, 759-765

Kristensen P, Andersen A, Irgens LM, et al. (1996) Cancer in offspring of parents engaged in agricultural activities in Norway: Incidence and risk factors in the farm environment. Int J Cancer **65**, 39-50

Kuhar MJ, Pert CB, Snyder SH (1973) Regional distribution of opiate receptor binding in monkey and human brain. Nature **245**, 447-450

Kung A (2008) Sex hormones and bone mineral density in men and women. Bone **43:Suppl. 1**, S24

Kunze B, Bürkle S, Kluba T (2009) Multifocal osteosarcoma in childhood. Musculoskelet Surg **93:1**, 27-31

Lai M, Wang SM, Zhang WL, et al. (2008) Effects of electroacupuncture on tumor growth and immune function in the Walker-256 model rat. Zhongguo Zhen Jiu **28:8,** 607-609

Lamont LA, Tranquilli WJ, Grimm KA (2000) Physiology of pain. Vet Clin N Am Sm Anim Pract **30**, 703-727

Langevin HM, Churchill DL, Cipolla MJ (2001) Mechanical signaling through connective tissue: a mechanism for the therapeutic effect of acupuncture. FASEB J **15**, 2275–2282

Langevin HM, Churchill DL, Wu J, et al. (2002) Evidence of connective tissue involvement in acupuncture. FASEB J **16:8**, 872-874

Langevin HM, Yandow JA (2002) Relationship of acupuncture points and meridians to connective tissue planes. Anat Rec **269:6**, 257-265

Lao LX, Zhang RX, Zhang G, et al. (2004) A parametric study of electroacupuncture on persistent hyperalgesia and Fos protein expression in rats. Brain Res **1020**, 18–29

Lau WK, Chan WK, Zhang JL, et al. (2008) Electroacupuncture inhibits cyclooxygenase-2 up-regulation in rat spinal cord after spinal nerve ligation. Neuroscience **155:2,** 463-8

Le Bars D, Gozariu M, Cadden SW (2001) Animal Models of Nociception. Pharmacol Rev **53:4,** 597-652

Le Bars D, Willer JC (2002) Pain modulation triggered by high-intensity stimulation: implication for acupuncture analgesia? Int Congress Ser **1238**, 11–29

Ledeboer A, Sloane EM, Milligan ED, et al. (2005) Minocycline attenuates mechanical allodynia and proinflammatory cytokine expression in rat models of pain facilitation. Pain **115**, 71–83

Lee BH (2002) Managing pain in human neonates--applications for animals. J Am Vet Med Assoc **221:2**, 233-237

Lee GS, Han JB, Shin MK, et al. (2003) Enhancement of electroacupuncture-induced analgesic effect in cholecystokinin-A receptor deficient rats. Brain Res Bull **62**, 161–164

Lee GS, Rho S, Shin MK, et al. (2002) The association of cholecystokinin-A receptor expression with the responsiveness of electroacupuncture analgesic effects in rat. Neurosci Lett **325,** 17–20

Lee H, Ernst E (2005) Acupuncture analgesia during surgery: a systematic review. Pain **114**, 511–517

Lee H, Schmidt K, Ernst E (2005) Acupuncture for the relief of cancer-related pain – a systematic review. Eur J Pain **9,** 437-444

Lee JH, Beitz AJ (1992) Electroacupuncture modifies the expression of c-fos in the spinal cord induced by noxious stimulation. Brain Res **577**, 80-91

Lee JH, Beitz AJ (1993) The distribution of brain-stem and spinal cord nuclei associated with different frequencies of electroacupuncture analgesia. Pain **52,** 11-28

Lee JH, Choi YH, Choi BT (2005) The anti-inflammatory effects of 2 Hz electroacupuncture with different intensities on acute carrageenan-induced inflammation in the rat paw. Int J Mol Med **16:1,** 99-102

Lee JH, Jang KJ, Lee YT, et al. (2006) Electroacupuncture inhibits inflammatory edema and hyperalgesia through regulation of cyclooxygenase synthesis in both peripheral and central nociceptive sites. Am J Chin Med **34:6,** 981-988

Lee MHM, Ernst M: Clinical and research observations on acupuncture analgesia and thermograph. In: Pomeranz B, Stux G (eds.) Scientific Bases of Acupuncture, Berlin: Spring-Verlag, 1989, pp. 157–176

Lei LG, Zhang YQ, Zhao ZQ (2004) Pain-related aversion and Fos expression in the central nervous system in rats. NeuroReport **15**, 67–71

Leonard A, Craft AW, Moss C, et al. (1996) Osteogenic sarcoma in the Rothmund-Thomson syndrome. Med Pediatr Oncol **26**, 249-253

Leong RJ, Chernow B (1988) The effects of acupuncture on operative pain and the hormonal responses to stress. Int Anesthesiol Clin **26:3,** 213-217

Leung A, Khadivi B, Duann JR et al. (2005) The effect of Ting point (tendinomuscular meridians) electroacupuncture on thermal pain: a model for studying the neuronal mechanism of acupuncture analgesia. J Altern Complement Med **11**, 653–661

Levine JD, Gormley J, Fields HL (1976) Observations on the analgesic effects of needle acupuncture (acupuncture). Pain **2**, 149–159

Lewith GT, Machin D (1983) On the evaluation of the clinical effects of acupuncture. Pain **16**, 111–127

Li A, Wang Y, Xin J, et al. (2007) Electroacupuncture suppresses hyperalgesia and spinal Fos expression by activating the descending inhibitory system. Brain Res **1186,** 171–179

Li AH, Hwang HM, Tan PP, et al. (2001) Neurotensin excites periaqueductal gray neurons projecting to the rostral ventromedial medulla. J Neurophysiol **85**, 1479–1488

Li AH, Zhang JM, Xie YK (2004) Human acupuncture points mapped in rats are associated with excitable muscle/skin-nerve complexes with enriched nerve endings. Brain Res **1012:1-2**,154-159

Li C, Wu P, Zhang L, et al. (2009) Osteosarcoma: limb salvaging treatment by ultrasonographically guided high-intensity focused ultrasound. Cancer Biol Ther **8:12**, 1102-1108

Li CH (1977) ß-endorphin: a pituitary peptide with potent morphine-like activity. Arch Biochem Biophys **183**, 592-604

Li CY, Zhu LX, Li WM, et al. (1993) Relationship between presynaptic depolarization and effect of acupuncture, γ-aminobutyric acid, opioid peptide substance P. [Article in Chinese, English abstract]. Acupunct Res **18**, 178–182

Li FP, Fraumeni JF, Mulvihill JJ, et al. (1988) A cancer family syndrome in twenty-four kindreds. Cancer Res **48**, 5358-5362

Li H, Ohishi H, Kinoshita A, et al. (1997) Localization of metobotropic glutamate receptor, mGluR7, in axon terminals of presumed nociceptive, primary afferent fibers in the superficial layer of the spinal dorsal horn: an electron microscope study in the rat. Neurosci Lett **233**, 153–156

Li J, Simone DA, Larson AA (1999) Windup leads to characteristics of central sensitization. Pain **79:1**, 75-82

Li WC, Hung DL, Kalnin A, et al. (2000) Brain activation of acupuncture induced analgesia. Neuroimage **11**, S701

Li Y, Tougas G, Chiverton S, et al. (1992) The effect of acupuncture on gastrointestinal function and disorders. Am J Gastroenterol **87**, 1372-1381

Lichtenstein L: Bone Tumors. St Louis: The C. V. Mosby Company, 1959

Liebeskind JC, Giesler GJ, Urca G: Evidence pertaining to an endogenous mechanism of pain inhibition in the central nervous system. In: Zotterman Y (ed.) Sensory Functions of the Skin in Primates. Oxford: Pergamon, 1976, pp. 561-573

Liebeskind JC, Guilbaud G, Besson JM, et al. (1973) Analgesia from electrical stimulation of the periaqueductal gray matter in the cat: behavioral observations and inhibitory effects on spinal cord interneurons. Brain Res **50**, 441-446

Lima D (1998) Anatomical basis for the dynamic processing of nociceptive input. Eur J Pain **2:3**, 195-202

Lin WW, Karin M (2007) A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest **117:5**, 1175-1183

Linde K, Allais G, Brinkhaus B, et al. (2009) Acupuncture for tension-type headache. Cochrane Database Syst Rev **1,** CD007587

Link MP, Eilber F: Osteosarcoma. In: Pizzo PA, Poplack DG (eds.) Principles and Practice of Pediatric Oncology (3rd ed.). Philadelphia: Lippincott-Raven, 1997, pp. 889-920

Link MP, Goorin AM, Horowitz M, et al. (1991) Adjuvant chemotherapy of high-grade osteosarcoma of the extremity. Updated results of the Multi-Institutional Osteosarcoma Study. Clin Orthop **270**, 8-14

Link MP, Goorin AM, Miser AW, et al. (1986) The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. N Engl J Med **314:25**, 1600-1606

Lipton A, Small E, Saad F, et al. (2002) The new bisphosphonate, Zometa (zoledronic acid), decreases skeletal complications in both osteolytic and osteoblastic lesions: a comparison to pamidronate. Cancer Invest **20:Suppl. 2**, 45-54

Liu B, Zhang RX, Wang L, et al. (2005) Effects of pertussis toxin on electroacupuncture-produced anti-hyperalgesia in inflamed rats. Brain Res **1044**, 87–92

Liu FY, Xing GG, Qu XX, et al. (2007) Roles of 5-hydroxytryptamine (5-HT) receptor subtypes in the inhibitory effects of 5-HT on C-fiber responses of spinal wide dynamic range neurons in rats. J Pharmacol Exp Ther **321**, 1046–1053

Liu H, Wang H, Sheng M, et al. (1994b) Evidence for presynaptic N-methyl-d-aspartate autoreceptors in the spinal cord dorsal horn. Proc Natl Acad Sci USA **91,** 8383–8387

Liu M, Wittbrodt E (2002) Low-dose oral naloxone reverses opioid-induced constipation and analgesia. J Pain Symptom Manage **23:1**, 48-53

Liu RH, Zhao ZQ (1992) Selective blockade by yohimbine of descending spinal inhibition from lateral reticular nucleus but not from locus coeruleus in rats. Neurosci Lett **142**, 65–68

Liu T (2009) Acupuncture: What Underlies Needle Administration? Evid Based Complement Alternat Med **6:2**, 185-193

Liu T (2009) Acupuncture: what underlies needle administration? Evid Based Complement Alternat Med **6:2**, 185-193

Liu WC, Hung DL, Kalnin A, et al. (2000) Brain activation of acupuncture induced analgesia. Neuroimage **11**, S710

Liu X (1996) The modulation of cerebral cortex and subcortical nuclei on NRM and their role in acupuncture analgesia. [Article in Chinese, English abstract]. Zhen Ci Yan Jiu **21**, 4–11

Liu X, Jiang MC, Huang PB, et al. (1990) Role of afferent C fibers in electroacupuncture of “zusanli” point in activating nucleus raphe magnus. [Article in Chinese, English abstract]. Acta Physiol Sin **42**, 523–533

Liu X, Zhu B, Zhang SX (1986) Relationship between electroacupuncture analgesia and descending pain inhibitory mechanism of nucleus raphe magnus. Pain **24**, 383–396

Liu Y, Varela M, Oswald R (1975) The correspondence between acupuncture points and motor points. Am J Chin Med **3**, 347-358

Lonardo F, Ueda T, Huvos AG, et al. (1997) p53 and MDM2 alterations in osteosarcomas: Correlation with clinicopathologic features and proliferative rate. Cancer **79**, 1541–1547

Looney A (2000) Using Acupuncture in Veterinary Practice. Veterinary Medicine **95:8**, 615-631

Lou H, Shen Y, Zou D, et al. (1990) A comparative study of the treatment of depression by electro-acupuncture. Acupunct Sci Int J **1**, 19-27

Lowney LI, Schulz K, Lowery PJ, et al. (1974) Partial purification of an opiate receptor from mouse brain. Science **183**, 749-753

Lu GW, Liang RZ, Xie JQ, et al. (1979) Role of peripheral afferent by needling point Zusanli. Sci Sin **22,** 680–692

Lu W, Dean-Clower E, Doherty-Gilman A, et al. (2008) The value of acupuncture in cancer care. Hematol Oncol Clin North Am **22:4**, 631-648

Luis Menéndez, Lucía Juárez, Eva García, et al. (2006) Analgesic effects of capsazepine and resiniferatoxin on bone cancer pain in mice. Neurosci Lett **393**, 70–73

Lund I, Lundeberg T (2008) Is it all about sex? Acupuncture for the treatment of pain from a biological and gender perspective. Acupuncture in Medicine **26:1**, 33-45

Lundeberg T (1984) The pain suppressive effect of vibratory stimulation and transcutaneous electrical nerve stimulation (TENS) as compared to aspirin. Brain Res **294**, 201–209

Lundeberg T, Lund I (2007) Are reviews based on sham acupuncture procedures in fibromyalgia syndrome (FMS) valid? Acupuncture in Medicine **25:3,** 100-106

Lundeberg T, Stener-Victorin E (2002) Is there a physiological basis for the use of acupuncture in pain? Int Congress Ser **1238,** 3–10

Ma J, Luo Y (2008) Effects of electroacupuncture on expressions of angiogenesis factors and anti-angiogenesis factors in brain of experimental cerebral ischemic rats after reperfusion. J Tradit Chin Med **28:3**, 217-222

Ma J, Yun-guang H, Zhang DH (2008) Effects of acupuncture on bone metabolism and serum estradiol level in ovariectomy-induced osteoporosis rats. [Article in Chinese, English abstract] Zhen Ci Yan Jiu **33:4**, 235-239

Ma JY, Zhao ZQ (2002) The involvement of glia in the long-term plasticity of spinal dorsal horn of rat. NeuroReport **13**, 1781–1784

MacPherson H, Asghar A (2006) Acupuncture needle sensations associated with De Qi: a classification based on experts' ratings. J Altern Complement Med **12**, 633–637

Madsen MV, Gøtzsche PC, Hróbjartsson A (2009) Acupuncture treatment for pain: systematic review of randomised clinical trials with acupuncture, placebo acupuncture, and no acupuncture groups. BMJ **338**, a3115

Magnusson M. Johansson K, Johansson B (1994) Sensory stimulation with electroacupuncture promotes normalization of postural control after stroke. Stroke J Cereb Circ **25**, 1176-1180

Mantyh PW, Clohisy DR, Koltzenburg M, et al. (2002) Molecular mechanisms of cancer pain. Nat Rev Cancer **2**, 201–209

Mao-Ying QL, Cui KM, Liu Q, et al. (2006) Stage dependent analgesia of electro-acupuncture in a mouse model of cutaneous cancer pain. Eur J Pain **10,** 689-694

Mark VH, Ervin FR, Yakovlev PI (1963) Stereotactic thalamotomy. III. The verification of anatomical lesion sites in the human thalamus. Arch Neurol **8**, 528–538

Marnett LJ, Kalgutkar AS (1999) Cyclooxygenase 2 inhibitors: discovery, selectivity and the future. Trends Pharmacol Sci **20**, 465-469

Masi L, Recenti R, Silvestri S, et al. (2007) Expression of cyclooxygenase-2 in osteosarcoma of bone. Appl Immunohistochem Mol Morphol **5:1**, 70-76

Mathews KA (2000) Pain assessment and general approach to management. Vet Clin North Am Sm Anim Pract **30**, 729-755

Mathews KA (2008) Neuropathic pain in dogs and cats: if only they could tell us if they hurt. Vet Clin North Am Small Anim Pract **38:6**, 1365-414

Mayer D, Price D, Raff A (1977) Antagonism of acupuncture analgesia to man by the narcotic antagonist naloxone. Brain Res **121**, 368-372

Mayer DJ (2000) Biological mechanisms of acupuncture. Prog Brain Res **122,** 457-477

Mayor DF (2007) Electroacupuncture: An introduction and its use for peripheral facial paralysis. JMC **84,** 1-19

Mazzaferro EM, Hackett TB, Stein TP, et al. (2001) Metabolic alterations in dogs with osteosarcoma. Am J Vet Res **62**, 1234-1239

McCall TL, Moore CP, Turk J, et al. (1989) Multilobular osteosarcoma of the mandible and orbit in a dog. Vet Pathol **26,** 92-94

McGuire SV, Vanable ED, McGuire MH, et al. (1991) Is there a link between fluoridated water and osteosarcoma? J Am Dent Assoc **122**, 38-45

McLennan H, Gilfillan K, Heap Y (1977) Some pharmacological observations on the analgesia induced by acupuncture in rabbits. Pain **3,** 229–238

McMahon SB (1996) NGF as a mediator of inflammatory pain. Philos Trans R Soc Lond B Biol Sci **351:1338**, 431-440

Melzack R (1996) Gate control theory: on the evolution of pain concepts. Pain Forum: Official Journal of the American Pain Society **5**, 128-138

Melzack R (1999) Pain – an overview. Acta Anaesthesiol Scand **43:9**, 880–884

Melzack R, Katz J: The Gate Control Theory: Reaching for the Brain. In: Hadjistavropoulos T, Craig KD (eds.) Pain: Psychological Perspectives. New Jersey: Lawrence Erlbaum Associates, 2003

Melzack R, Wall PD (1965) Pain mechanisms: a new theory. Science **150:699**, 971–979

Melzack R, Wall PD (1984) Acupuncture and transcutaneous electrical nerve stimulation. Postgrad Med J **60**, 893-896

Menétrey D, Besson JM (1982) Electrophysiological characteristics of dorsal horn cells in rats with cutaneous inflammation resulting from chronic arthritis. Pain **13:4**, 343-364

Mense S, Hoheisel V, Kaske A, et al. (1997) Muscle pain: Basic mechanisms and clinical correllates. Chapter 32 in Jensen TS, Turner JA, Wiesenfeld-Hallin Z (eds): Proceedings of the 8th World Congress in Pain Research and Management **8,** 479-496

Mense SS (2004) Functional neuroanatomy for pain stimuli. Reception, transmission, and processing. [Article in German, English abstract]. Schmerz **18:3,** 225-37

Mercadante S (1997) Malignant bone pain: pathophysiology and treatment. Pain **69**, 1–18

Merskey H (1968) Psychological aspects of pain. Postgrad Med J **44**, 297-306

Merskey H, Spear FG: Pain, psychological and psychiatric aspects. London: Bailliere, Tindall & Cassell, 1967

Merskey HM, Bogduk N: Classification of Chronic Pain. 2nd ed. IASP Press, Seattle, 1994, p.211

Messerschmitt PJ, Garcia RM, Abdul-Karim FW, et al. (2009) Osteosarcoma. J Am Acad Orthop Surg **17:8**, 515-527

Meyer RA, Ringkamp M, Campbell JN, et al.: Peripheral mechanisms of cutaneous nociception. In: Wall P, Melzack R (eds.) Textbook of Pain. 5th ed. New York: Elsevier, 2005, pp. 1–23

Meyr AJ, Saffran B (2008) The pathophysiology of the chronic pain cycle. Clin Podiatr Med Surg **25:3**, 327-346

Meyr AJ, Steinberg JS (2008) The physiology of the acute pain pathway. Clin Podiatr Med Surg **25:3**, 305-326

Millan MJ (1999) The induction of pain: an integrative review. Prog Neurobiol **57**, 1–164

Millan MJ (2002) Descending control of pain. Prog Neurobiol **66**, 355–474

Miller RW, Boice JD Jr, Curtis RE: Bone Cancer. In: Schottenfeld D, Fraumeni JF, Editors. Cancer Epidemiology and Prevention. 2nd ed. New York: Oxford University Press, 1996, pp. 971-983

Minton O, Higginson IJ (2007) Electroacupuncture as an adjunctive treatment to control neuropathic pain in patients with cancer. J Pain Symptom Manag **33:2**, 115-117

Misdorp W, Hart AA (1979) Some prognostic and epidemiologic factors in canine osteosarcoma. J Natl Cancer Inst **62**, 537-545

Miser AW, Chayt KJ, Sandlund JT, et al. (1986) Narcotic withdrawal syndrome in young adults after the therapeutic use of opiates. Am J Dis Child **140:6**, 603-604

Moalic S, Liagre B, Le Bail JC, et al. (2001) Dose-dependent modulation of apoptosis and cyclooxygenase-2 expression in human 1547 osteosarcoma cells by NS-398, a selective cyclooxygenase-2 inhibitor. Int J Oncol **18**, 533-540

Mogil JS (1999) The genetic mediation of individual differences in sensitivity to pain and its inhibition. Proc Natl Acad Sci USA **96**, 7744–7751

Mohammed SI, Coffman K, Glickman NW, et al. (2001) Prostaglandin E2 concentrations in naturally occurring canine cancer. Prostaglandins Leukot Essent Fatty Acids **64**, 1-4

Mohammed SI, Khan KN, Sellers RS, et al. (2004) Expression of cyclooxygenase-1 and 2 in naturally-occurring canine cancer. Prostaglandins Leukot Essent Fatty Acids **70**, 479-483

Moss ME, Kanarek MS, Anderson HA, et al. (1995) Osteosarcoma, seasonality and environmental factors in Wisconsin, 1979-1989. Arch Environ Health **50**, 235-241

Mullins MN, Lana SE, Dernell WS, et al. (2004) Cyclooxygenase-2 expression in canine appendicular osteosarcomas. J Vet Intern Med **18:6,** 859-865

Napadow V, Ahn A, Longhurst J, et al. (2008) The status and future of acupuncture clinical research. J Altern complement Med **14:7**, 861-869

Nappi G, Facchinetti F, Legnante G, et al. (1982) Different releasing effects of traditional manual acupuncture and electro-acupuncture on proopiocortin-related peptides. Acupunct Electrother Res **7:2-3**, 93-103

Naruse T, Nishida Y, Hosono K, et al. (2006) Meloxicam inhibits osteosarcoma growth, invasiveness and metastasis by COX-2-dependent and independent routes. Carcinogenesis **27:3,** 584-592

Newton WA, Meadows AT, Shimada H, et al. (1991) Bone sarcomas as a second malignant neoplasm following childhood cancer. Cancer **67**, 193-201

Niu WX, He GD, Liu H, et al. (2007) Effects and probable mechanisms of electroacupuncture at the Zusanli point on upper gastrointestinal motility in rabbits. J Gastroenterol Hepatol **22:10**, 1683-1689

Obata K, Noguchi K (2004) MAPK activation in nociceptive neurons and pain hypersensitivity. Life Sci **74**, 2643–2653

O'Brien MG, Straw RC, Withrow SJ, et al. (1993) Resection of pulmonary metastases in canine osteosarcoma. Vet Surg **22:2**, 105-109

Ogilvie GK: Bone tumors. In: Rosenthal R (ed.) Veterinary Oncology Secrets. Philadelphia: Hanley and Belfus Inc., 2001, pp. 139-146

Ohsawa H, Okada K, Nishijo K, et al. (1995) Neural mechanism of depressor responses of arterial pressure elicited by acupuncture-like stimulation to a hindlimb in anesthetized rats. J Auton Nerv Syst **51:1**, 27-35

Okada K, Oshima M, Kawakita K (1996) Examination of the afferent fiber responsible for the suppression of the jaw-opening reflex in heat, cold, and the manual acupuncture stimulation in rats. Brain Res **740**, 201–207

Operskalski EA, Preston-Martin S, Henderson BE, et al. (1987) A case-control study of osteosarcoma in young persons. Am J Epidemiol **126**,118-126

Ørstavik K, Weidner C, Schmidt R, et al. (2003) Pathological C-fibres in patients with a chronic painful condition. Brain **126:3**, 567-578

Osoba D, Tannock I, Ernst D, et al. (1999) Health-related quality of life in men with metastatic prostate cancer treated with prednisone alone or mitoxantrone and prednisone. J Clin Oncol **17:6**, 1654-1663

Otto KC, Quinn C, Sung YF (1998) Auricular acupuncture as an adjunctive treatment for cocaine addiction, a pilot study. Am J Addict **7**, 164-170

Pacharinsak C**,** Beitz A (2008) Animal models of cancer pain. Comp Med **58:3,** 220-233

Pan B, Castro-Lopes JM, Coimbra A (1996) Activation of anterior lobe corticotrophs by electroacupuncture or noxious stimulation in the anaesthetized rat, as shown by co-localization of Fos protein with ACTH and β-endorphin and increased hormone release. Brain Res Bull **40**, 175–182

Pan B, Castro-Lopes JM, Coimbra A (1997) Chemical sensory deafferentiation abolishes hypothalamic pituitary activation induced by noxious stimulation or electroacupuncture but not only decreases that caused by immobilization stress. A c-fos Study. Neuroscience **78**, 1059–1068

Pancrazio J, Kamatchi G, Roscoe A, et al. (1998) Inhibition of neuronal Na+ channels by antidepressant drugs. J Pharmacol Exp Ther **284:1**, 208-214

Pariente J, White P, Frackowiak RS, et al. (2005) Expectancy and belief modulate the neuronal substrates of pain treated by acupuncture. Neuroimage **25**, 1161–1167

Park SB, Krishnan AV, Lin CS, et al. (2008) Mechanisms underlying chemotherapy-induced neurotoxicity and the potential for neuroprotective strategies. Curr Med Chem **15:29**, 3081-3094

Parwatikar S, Brown M, Stern J, et al. (1979) Acupuncture, hypnosis and experimental pain. I. Study with volunteers. Acupunct Electrother Res Int J **3**,161-190

Patnaik AK (1990) Canine extraskeletal osteosarcoma/chondrosarcoma: a clinicopathologic study of 14 cases. Vet Pathol **27**, 46-55

Patnaik AK, Lieberman PH, Liu SK (1989) Canine sinonasal skeletal neoplasms: chondrosarcomas and osteosarcomas. Vet Pathol **21**, 475-482

Pedersen LH, Blackburn-Munro G (2006) Pharmacological characterisation of place escape/avoidance behaviour in the rat chronic constriction injury model of neuropathic pain. Psychopharmacology (Berl) **185:2**, 208-217

Peets J, Pomeranz B (1978) CXBK mice deficient in opiate receptors show poor electroacupuncture analgesia. Nature **273**, 675–676

Peltier LF: Tumors of bone and soft tissues. In: Orthopedics: A History and Iconography. San Francisco, CA: Norman Publishing, 1993

Peng A, Greenfield WA (1990) Precise scientific explanation of acupuncture mechanisms: are we on the threshold? Acupunct Sci Int J **1**, 28-29

Pert C, Snyder S (1973) Opiate receptors: demonstration on nervous tissue. Science **170**, 1011-1014

Pert CB, Ervin FR, Snyder SH (1976) Opiate receptors: autoradiographic localization in rat brain. Proc Natl Acad Sci USA **73**, 3729–3733

Pignatti G, Bacci G, Picci P, et al. (1991) Telangiectatic osteogenic sarcoma of the extremities. Results in 17 patients treated with neoadjuvant chemotherapy. Clin Orthop Relat Res **270**, 99-106

Pittler MH, Ernst E (2008) Complementary therapies for neuropathic and neuralgic pain: systematic review. Clin J Pain **24:8,** 731-733

Pomeranz B (1978) Do endorphins mediate acupuncture analgesia? Adv Biochem Psychopharmacol **18**, 351-359

Pomeranz B, Cheng R (1979) Suppression of noxious responses in single neurons of cat spinal cord by electroacupuncture and its reversal by the opiate antagonist naloxone. Exp Neurol **64**, 327–341

Pomeranz B, Chiu D (1976) Naloxone blockade of acupuncture analgesia: endorphin implicated. Life Sci **19**, 1757–1762

Pomeranz B, Nguyen P (1987) Intrathecal diazepam suppresses nociceptive reflexes and potentiates electroacupuncture effects in pentobarbitol-anesthetized rats. Neurosci Lett **77**, 316–320

Pomeranz B, Paley D (1979) Electroacupuncture hypolgesia is mediated by afferent nerve impulses: an electrophysiological study in mice. Exp Neurol **66**, 398–402

Pomeranz B: Acupuncture research related to pain, drug addiction and nerve regeneration. In: Pomeranz B, Stux G (eds.) Scientific Basis of Acupuncture. Berlin, Springer-Verlag, 1989

Pomeranz B: Scientific basis of acupuncture. In: Stux G, Hammerschlag R (eds.) Clinical acupuncture: Scientific Basis. Heidelberg, Germany: Springer, 2001, pp. 197–199

Pool RR: Tumors of bone and cartilage. In: Moulton JE (ed.) Tumors in Domestic Animals. Berkley: University of California Press, 1990, pp. 157-230

Porges P (1988) Cancer pain. [Article in German, English abstract]. Schmerz **2:2**, 59-65

Portenoy RK, Lesage P (1999) Management of cancer pain, Lancet **353**, 1695–1700

Porter A, McEwan A, Powe J, et al. (1993) Results of a randomized phase-III trial to evaluate the efficacy of strontium-89 adjuvant to local field external beam irradiation in the management of endocrine resistant metastatic prostate cancer. Int J Radiat Oncol Biol Phys **25:5**, 805-813

Price DD (2000) Psychological and neural mechanisms of the affective dimension of pain. Science **288**, 1769–1772

Price DD, Rafii A, Watkins LR, et al. (1984) A psychophysical analysis of acupuncture analgesia. Pain **19**, 27–42

Provisor AJ, Ettinger LJ, Nachman JB, et al. (1997) Treatment of nonmetastatic osteosarcoma of the extremity with preoperative and postoperative chemotherapy: a report from the Children's Cancer Group. J Clin Oncol **15:1**, 76-84

Przewlocki R, Costa T, Lang J, et al. (1987) Pertussis toxin abolishes the antinociception mediated by opioid receptors in rat spinal cord. Eur J Pharmacol **144,** 91–95

Pui CH, Dodge RK, George SL, et al. (1987) Height at diagnosis of malignancies. Arch Dis Child **62**, 495-499

Pypendop BH, Ilkiw JE (2008) Pharmacokinetics of tramadol, and its metabolite O-desmethyl-tramadol, in cats. J Vet Pharmacol Ther **31:1**, 52-59

Quilty P, Kirk D, Bolger J, et al. (1994) A comparison of the palliative effects of strontium-89 and external beam radiotherapy in metastatic prostate cancer. Radiother Oncol **31:1**, 33-40

Regan JM, Peng P (2000) Neurophysiology of cancer pain. Cancer Control **7**:111–119

Reisner L (2003) Antidepressants for chronic neuropathic pain. Curr Pain Headache Rep **7:1**, 24-33

Ren K, Hylden JLK, Williams GM, et al. (1992) The effects of a non-competitive NMDA receptor antagonist, MK-801, on behavioral hyperalgesia and dorsal horn neuronal activity in rats with unilateral inflammation. Pain **50**, 331–344

Reston J (1971) Now, about my operation in Peking; Now, let me tell you about my appendectomy in Peking.... New York Times **July 26**, p. 1 and 6

Reynolds DV (1969) Surgery in the rat during electrical analgesia induced by focal brain stimulation. Science **194**, 444–445

Richardson H, Vincent CA (1986) Acupuncture for the treatment of pain: a review of evaluative research. Pain **24**, 15–40

Ries LAG, Smith MA, Gurney JG, et al. “Cancer incidence and survival among children and adolescents: United States SEER program 1975-1995”. Bethesda, Md: National Cancer Institute; 1999 NIH Pub No 99–4649 Available at http://seer.cancer.gov/publications/childhood/. Accessed March 21 and August 10, 2008

Romita VV, Suk A, Henry JL (1997) Parametric study on electroacupuncture like stimulation in a rat model: effects of intensity, frequency, and duration of stimulation on evoked antinociception. Brain Res Bull **42**, 289–296

Rosen G, Caparros B, Huvos AG, et al. (1982) Preoperative chemotherapy for osteogenic sarcoma: selection of postoperative adjuvant chemotherapy based on the response of the primary tumor to preoperative chemotherapy. Cancer **49:6**, 1221-1230

Rosen G, Forscher CA, Mankin HJ, et al. “Neoplasms of the Bone and Soft Tissue.” In: Holland and Frei (eds.): Cancer Medicine e.5 American Cancer Society; 2000 Available at http://www.cancer.org/downloads/PUB/DOCS/SECTION35/121pdf. Acessed June 23, 2009

Rottmann S, Jung K, Vohn R, et al. (2009) Long-term depression of pain-related cerebral activation in healthy man: An fMRI study. Eur J Pain **Nov 5** [Epub ahead of print]

Rowbotham M, Harden N, Stacey B, et al. (1998) Gabapentin for the treatment of postherpetic neuralgia: a randomized controlled trial. JAMA **280:21**, 1837-1842

Ru G, Terracini B, Glickman LT (1998) Host related risk factors for canine osteosarcoma. Vet J **156**, 31-39

Rugh R: Reproductive systems of adult mice – The adult female; F. The estrous cycle. In: The Mouse: Its Reproduction and Development. New York: Oxford University Press, 1990, pp. 38-42

Rutkow IM: The nineteenth century. In: Surgery: An Illustrated History. St Louis, Mo: Mosby-Year Book, 1993, 321-504

Saab CY, Shamaa F, El Sabban ME, et al. (2009) Transient increase in cytokines and nerve growth factor in the rat dorsal root ganglia after nerve lesion and peripheral inflammation. J Neuroimmunol **208:1-2**, 94-103

Saad F, Gleason D, Murray R, et al. (2002) A randomized, placebo-controlled trial of zoledronic acid in patients with hormone-refractory metastatic prostate carcinoma. J Natl Cancer Inst **94:19**, 1458-1468

Sabino MA, Ghilardi JR, Jongen JL, et al. (2002) Simultaneous reduction in cancer pain, bone destruction, and tumor growth by selective inhibition of cyclooxygenase 2. Cancer Res **62**, 7343–7349

Sager M (1993) Zur Problematik der Quantifizierung von Schmerzen beim Tier. [Article in German] Berl Münch Tierärztl Wschr **106**, 289-293

Sandkuhler J, Fu QG, Helmchen C (1990) Spinal somatostatin superfusion in vivo affects activity of cat nociceptive dorsal horn neurons: comparison with spinal morphine. Neuroscience **34**, 565–576

Sanerkin NG (1980) Definitions of osteosarcoma, chondrosarcoma, and fibrosarcoma of bone. Cancer **46:1**, 178-185

Sanford J, Ewbank R, Molony V, et al., (1986) Guidelines for the recognition and assessment of pain in animals. Vet Rec **118**, 334-338

Sarrau S, Jourdan J, Dupuis-Soyris F, et al. (2007) Effects of postoperative ketamine infusion on pain control and feeding behaviour in bitches undergoing mastectomy. J Small Anim Pract **48:12**, 670-676

Sarton E, Olofsen E, Romberg R, et al. (2000) Sex differences in morphine analgesia: an experimental study in healthy volunteers. Anesthesiology **93:5**, 1245-1254

Schmidt K, Eland J, Weiler K (1994) Pediatric cancer pain management: a survey of nurses' knowledge. J Pediatr Oncol Nurs **11:1**, 4-12

Schwartz C: Four Paws Five Directions. A Guide to Chinese Medicine for Cats and Dogs. Berkeley, CA: Celestial Arts Publishing, 1996

Schwartzbaum JA, George SL, Pratt CB, et al. (1991) An exploratory study of environmental and medical factors potentially related to childhood cancer. Med Pediatr Oncol **19**, 115-121

Schwarz E (1966) 1st anniversary of the "International Association of Electroacupuncture". Zahnarztl Prax **17:23**, 280

Scully SP, Ghert MA, Zurakowski D, et al. (2002) Pathologic fracture in osteosarcoma : prognostic importance and treatment implications. J Bone Joint Surg Am **84-A:1**, 49-57

Sekido R, Ishimaru K, Sakita M (2003) Differences of electroacupuncture-induced analgesic effect in normal and inflammatory conditions in rats. Am J Chin Med **31**, 955–965

Selvarajah GT, Kirpensteijn J, van Wolferen ME, et al. (2009) Gene expression profiling of canine osteosarcoma reveals genes associated with short and long survival times. Mol Cancer **8**, 72

Serafini A, Houston S, Resche I, et al. (1998) Palliation of pain associated with metastatic bone cancer using samarium-153 lexidronam: a double-blind placebo-controlled clinical trial. J Clin Oncol **16:4**, 1574-1581

Setoguchi A, Sakai T, Okuda M, et al. (2001) Aberrations of the P53 tumor suppressor gene in various tumors in dogs. Am J Vet Res **62**, 433-439

Sevcik MA, Ghilardi JR, Peters CM, et al. (2005) AntiNGF therapy profoundly reduces bone cancer pain and the accompanying increase in markers of peripheral and central sensitization. Pain **115**, 128–141

Sheehan DK, Webb A, Bower D, et al. (1992) Level of cancer pain knowledge among baccalaureate student nurses. J Pain Symptom Manage **7:8**, 478-484

Shen E, Cai TD, Lan Q (1974) Effects of supraspinal structures on acupuncture-induced inhibition of visceral-somatic reflex. Chin J Med **10,** 628–633

Shen E, Cai TD, Lan Q (1975) Supraspinal participation in the inhibitory effect of acupuncture on visceral-somatic reflex discharges. Chin J Med **1,** 431–440

Shen E, Ma WH, Lan Q (1978) Involvement of descending inhibition in the effect of acupuncture on the splanchnically evoked potential in the orbital cortex of cat. Sci Sin **21**, 677–685

Shen E, Wu WY, Du HJ, et al. (1973) Electromyographic activity produced locally by acupuncture manipulation. Chin Med J **9**, 532–535

Shen S, Li J, Wang XM, et al. (1996) Angiotensin II and anti-electroacupuncture analgesia in the spinal cord. [Article in Chinese, English abstract]. Acta Physiol Sin **48**, 543–550

Sheng LC, Chang TH (1960) Electroacupuncture anesthesia in oral surgery: a preliminary report. Chin Med J **80**, 97-99

Sheng LL, Nishiyama K, Honda T, et al. (2000) Suppressive effects of Neiting acupuncture on toothache: an experimental analysis on Fos expression evoked by tooth pulp stimulation in the trigeminal subnucleus pars caudalis and the periaqueductal gray of rats. Neurosci Res **38**, 331–339

Shenoy R, Pillai, A, Sokhi K, et al. (2008) Survival trends in osteosarcoma of humerus. Eur J Cancer Care **17:3**, 261-269

Shimo T, Matsumura S, Ibaragi S, et al. (2007) Specific inhibitor of MEK-mediated cross-talk between ERK and p38 MAPK during differentiation of human osteosarcoma cells. J Cell Commun Signal **1:2,** 103-111

Siebert K, Zhang Y, Leahy K, et al. (1997) Distribution of COX-1 and COX-2 in normal and inflamed tissues. Adv Exp Med Bio **400A**, 167-170

Siedentopf CM, Golaszewski SM, Mottaghy FM, et al. (2002) Functional magnetic resonance imaging detects activation of the visual association cortex during laser acupuncture of the foot in humans. Neurosci Lett **327**, 53–56

Simon RH, Arbo TE (1986) Morphine increases metastatic tumor growth. Brain Res Bull **16:3**, 363-7

Sims J (1997) The mechanism of acupuncture analgesia: a review. Complement Ther Med **5**, 102–111

Sjölund B, Terenius L, Ericksson M (1977) Increased cerebrospinal fluid levels of endorphin after electroacupuncture. Acta Physiol Scand **100**, 382-384

Slayter MV, Boosinger TR, Pool RR et al. (1994) Histological Classification of Bone and Joint Tumors of Domestic Animals. American Registry of Pathology and the World Health Organization Collaborating Center for Comparative Oncology, Washington, D.C., Armed Forces Institute of Pathology

Smith WC, Bourne D, Squair J, et al. (1999) A retrospective cohort study of post mastectomy pain syndrome. Pain **83:1**, 91-95

Snyder SH (1975) Opiate receptor in normal and drug altered brain function. Nature (Lond) **257**, 185-189

Song L, Zhu ZH, Duan XL, et al. (2006) Effects of electroacupuncture at “Zusanli” (ST 36) on ERK1/2 phosphorylation in the dorsal horn of spinal cord of the rat. [Article in Chinese, English abstract]. Zhongguo Zhen Jiu **26**, 362–366

Song P, Hu JY, Zhao ZQ (2002) Spinal somatostatin SSTR2A receptors are preferentially up-regulated and involved in thermonociception but not mechanonociception. Exp Neurol **178**, 280–287

Song P, Zhao ZQ (2001) Involvement of glia in the development of morphine tolerance. Neurosci Res **39**, 281–286

Song XJ, Zhao ZQ (1993a) Differential effects of NMDA and non-NMDA receptor antagonists on spinal cutaneous vs. muscular nociception in the cat. NeuroReport **4**, 17–20

Song XJ, Zhao ZQ (1993b) NMDA and non-NMDA receptors mediating nociceptive and non-nociceptive transmission in spinal cord of cat. Acta Pharmacol Sin **14**, 481–485

Staud R, Craggs JG, Robinson ME, et al. (2007) Brain activity related to temporal summation of C-fiber evoked pain. Pain **129**, 130–142

Staud R, Price DD (2006) Mechanisms of acupuncture analgesia for clinical and experimental pain. Exp Rev of Neurother **6:5**, 661-667

Steegers MA, Snik DM, Verhagen AF, et al. (2008) Only half of the chronic pain after thoracic surgery shows a neuropathic component. J Pain **9:10**, 955-961

Stein C (1991) Peripheral analgesic actions of opioids. J Pain Symptom Manage **6**, 119–124

Stein C, Schäfer M, Machelska H (2003) Attacking pain at its source: new perspectives on opioids. Nat Med **9**, 1003–1008

Steiss JE, White NA, Bowen JM (1989) Electro-acupuncture in the treatment of chronic lameness in horses and ponies: a controlled clinical trial. Can J Vet Res **53**, 239-243

Stephenson N, Dalton JA, Carlson J, et al. (2009) Racial and ethnic disparities in cancer pain management. J Natl Black Nurses Assoc **20:1**, 11-18

Sternberg WF, Liebeskind JC (1995) The analgesic response to stress: genetic and gender considerations. Eur J Anaesthesiol Suppl **10**, 14-17

Steverson S, Hohn RB, Pohler OEM, et al. (1982) Fracture-associated sarcoma in the dog. JAVMA **180:10**, 1189-1196

Stollberg G: Acupuncture in Western Europe. In: Thieme Almanack 2007: Acupuncture and Chinese Medicine. Germany: Georg Thieme Verlag KG, 2007, pp. 137-150

Straw RC: Tumors of the skeletal system. In: Withrow SJ, MacEwen EG (eds.) Small Animal Clinical Oncology. 2nd edition. Philadelphia: WB Saunders Company, 1996, pp. 287-315

Strube P, Mehta M, Baerenwaldt A, et al. (2009) Sex-specific compromised bone healing in female rats might be associated with a decrease in mesenchymal stem cell quantity. Bone **45:6**, 1065-1072

Sun RQ, Wang HC, Wan Y, et al. (2004) Suppression of neuropathic pain by peripheral electrical stimulation in rats: A-opioid receptor and NMDA receptor implicated. Exp Neurol **187**, 23–29

Sun S, Cao H, Han M, et al. (2007a) New evidence for the involvement of spinal fractalkine receptor in pain facilitation and spinal glial activation in rat model of monoarthritis. Pain **129**, 64–75

Sun S, Chen WL, Wang PF, et al. (2006) Disruption of glial function enhances electroacupuncture analgesia in arthritic rats. Exp Neurol **198**, 294–302

Sun S, Mao-Ying QL, Cao H, et al. (2007b) Is functional state of spinal microglia involved in the anti-allodynic and anti-hyperalgesic effects of electroacupuncture in rat model of monoarthritis? Neurobiol Dis **26**, 558–568

Sun Y, Gan TJ, Dubose JW, et al. (2008) Acupuncture and related techniques for postoperative pain: a systematic review of randomized controlled trials. Br J Anaesth **101:2**, 151-60

Sun Y, Gan TJ. (2008) Acupuncture for the management of chronic headache: a systematic review. Anesth Analg **107:6**, 2038-2047

Sun Z, Li X, Su Z, et al. (2009) Electroacupuncture-enhanced differentiation of bone marrow stromal cells into neuronal cells. J Sport Rehabil **18:3**, 398-406

Sydenham T: Tractatus de podagra et hydrope. Geneva, 1686 In: Latham RG. The Works of Thomas Sydenham, MD. 3rd ed., Vol. 2. Birmingham, AL: Classics of Medicine Library, 1979

Ta HT, Dass CR, Choong PFM, et al. (2009) Osteosarcoma treatment: state of the art. Cancer Metastasis Rev **28**, 247–263

Taguchi R (2008) Acupuncture anesthesia and analgesia for clinical acute pain in Japan. Evid Based Complement Alternat Med **5:2**, 153-158

Takakura N, Ogawa H, Iijima S, et al. (1995) Effect of acupuncture at the Hoku point on vibration-induced finger flexion reflex in man: comparison between press needle technique, electroacupuncture, and in-situ technique. Am J Chin Med **23**, 313–318

Takeshige C, Oka K, Mizuno T, et al. (1993) The acupuncture point and its connecting central pathway for producing acupuncture analgesia. Brain Res Bull **30**, 53–67

Takeshige C, Sato M (1996) Comparisons of pain relief mechanisms between needling to the muscle, static magnetic field, external qigong and needling to the acupoint. Acupunct Electrother Res **21:2,** 119-131

Takeshige C, Sato T, Mera T, et al. (1992) Descending pain inhibitory system involved in acupuncture analgesia. Brain Res Bull **29**, 617–634

Takeshige C, Tsuchiya M, Guo SY, et al. (1991) Dopaminergic transmission in the hypothalamic arcuate nucleus to produce acupuncture analgesia in correlation with the pituitary gland. Brain Res Bull 26, 113–122

Takeshige C: Mechanism of acupuncture analgesia based animal experiment. In: Pomeranz B, Stux G (eds.) Scientific Bases of Acupuncture. Berlin: Springer-Verlag, 1989, pp. 53–78

Tang NM, Dong HW, Wang XM, et al. (1997) Cholecystokinin antisense RNA increases the analgesic effect induced by electroacupuncture or low dose morphine: conversion of low responder rats into high responders. Pain **71**, 71–80

Tang YH, Pan YZ, Liu GJ, et al. (1988) Gamma—aminobutyric acid and acupuncture analgesia. [Article in Chinese, English abstract]. J Norman Bethune Univ Med Sci **14**, 490–492

Tchekalarova J, Pechlivanova D, Kambourova T, et al. (2003) The effects of sarmesin, an Angiotensin II analogue on seizure susceptibility, memory retention and nociception. Regul Pept **111**, 191–197

Teh CF, Zaslavsky AM, Reynolds CF 3rd, et al. (2009) Effect of Depression Treatment on Chronic Pain Outcomes. Psychosom Med **Oct 29** [Epub ahead of print]

Ter Reit G, Kleinjen J, Knipschild P (1990) Meta-analysis into the effect of acupuncture on addiction. Br J Gen Pract **40**, 379-382

Terenius L (1973) Characteristics of the "receptor" for narcotic analgesics in synaptic plasma membrane fraction from rat brain. Acta Pharmacol et Toxicol **33**, 377-384

Tershner SA, Helmstetter FJ (2000) Antinociception produced by mu opioid receptor activation in the amygdala is partly dependent on activation of mu opioid and neurotensin receptors in the ventral periaqueductal gray. Brain Res **865**, 17–26

Teschemacher H, Opheim KE, Cox BM, et al. (1975) A peptide-like substance from pituitary that acts like morphine. 1. Isolation. Life Sci **16**, 1771-1776

Theilen GH, Madewell BR: Tumors of the skeleton. In: Theilen GH, Madewell BR (eds.) Veterinary Cancer Medicine. Philadelphia: Lea and Febiger, 1987, pp.471-493

Thomas R, Wang HJ, Tsai PC, et al. (2009) Influence of genetic background on tumor karyotypes: evidence for breed-associated cytogenetic aberrations in canine appendicular osteosarcoma. Chromosome Res **17:3**, 365-377

Thrall DE, Goldschmidt MH, Biery DN (1981) Malignant tumor formation at the site of previously irradiated acanthomatous epulides in four dogs. JAVMA **178:2**, 127-132

Toda K (2002) Afferent nerve characteristics during acupuncture stimulation. Int Congress Ser **1238,** 49–61

Toda K, Ichioka M (1978) Electroacupuncture: relations between forelimb afferent impulses and suppression of jaw-opening reflex in the rat. Exp Neurol **61**, 465–470

Todd EM, Kucharski A: Pain: Historical Perspectives. In: Warfield CA, Bajwa ZH (eds.) Principles and Practice of Pain Medicine. 2nd ed. McGraw Hill, 2004

Toma N, Sgambato V, Couture R (1997) Effect of angiotensin II on a spinal nociceptive reflex in the rat: receptor and mechanism of action. Life Sci **61**, 503–513

Tough EA, White AR, Cummings TM, et al. (2009) Acupuncture and dry needling in the management of myofascial trigger point pain: A systematic review and meta-analysis of randomised controlled trials. Eur J Pain **13:1**, 3-10

Tracey KJ (2007) Physiology and immunology of the cholinergic antiinflammatory pathway. J Clin Invest **117:2**, 289-296

Tucker MA, D'Angio GJ, Boice JD, et al. (1987) Bone sarcomas linked to radiotherapy and chemotherapy in children. New Eng J Med **317**, 588-593

Turini M, DuBois R (2002) Cyclooxygenase-2: a therapeutic target. Annu Rev Med **53**, 35-57

Turrel JM, Pool RR (1982) Primary bone tumors in the cat: A retrospective study of 15 cats and a literature review. Vet Radiol **23,** 152-166

Uchida Y, Nishigori A, Takeda D, et al. (2003) Electroacupuncture induces the expression of Fos in rat dorsal horn via capsaicin-insensitive afferents. Brain Res **978**, 136–140

Ulett G (1983) Acupuncture is not hypnosis. Am J Acupunct **11**, 5-13

Ulett G, Han S, Han JS (1998a) Electroacupuncture: mechanisms and clinical application. Biol Psychiatry **44**, 129-138

Ulett GA (1996) Conditioned healing with electroacupuncture. Altern Ther Health Med **2**, 56-60

Ulett GA, Han JS, Han S (1998b) Traditional and Evidence-Based Acupuncture: History, Mechanisms, and Present Status. South Med J **91:12**, 1115-1120

Ulett GA: Studies supporting the concept of physiological acupuncture. In: Pomeranz B, Stux G (eds.) Scientific Bases of Acupuncture. Berlin: Spring-Verlag, 1989, pp. 177–198

Underwood WJ (2002) Pain and distress in agricultural animals. J Am Vet Med Assoc **221:2**, 208-211

Ungerstedet U (1971) Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol Scad **83:Suppl. 367**, 1–48

Vadivelu N, Schreck M, Lopez J, et al. (2008) Pain after mastectomy and breast reconstruction. Am Surg **74:4**, 285-296

Van Remoortere MP, Meert TF, Vissers KC, et al. (2007) Refinement of symptoms of neuropathic pain measurements after various transections of the nerve endings of the sciatic and femoral nerve in rats: an exploratory behavioral analysis. Anesth Analg **104:5**, 1236-1245

van Wilgen CP, Dijkstra PU, van der Laan BF, et al. (2004) Morbidity of the neck after head and neck cancer therapy. Head Neck **26:9**, 785-791

Vane JR, Bakhle YS, Botting RM (1998) Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol **38**, 97-120

VanWormer AM, Lindquist R, Sendelbach SE. (2008) The effects of acupuncture on cardiac arrhythmias: a literature review. Heart Lung **37:6**, 425-431

Vardanyan A, Wang R, Vanderah TW, et al. (2009) TRPV1 receptor in expression of opioid-induced hyperalgesia. J Pain **10:3**, 243-252

Vieth I: The Yellow Emperor's Classic of Internal Medicine. Berkeley, CA: University of California Press, 1949, pp 260

Viganó A, Bruera E, Suarez-Almazor ME (1998) Age, pain intensity, and opioid dose in patients with advanced cancer. Cancer **83**, 1244-1250

Vincent CA, Richardson H (1986) The evaluation of therapeutic acupuncture: concepts and methods. Pain **24**, 1–13

Visser E, Schug SA (2006) The role of ketamine in pain management. Biomed Pharmacother **60:7**, 341-348

Wacnik PW, Baker CM, Herron MJ, et al. (2005a) Tumor-induced mechanical hyperalgesia involves CGRP receptors and altered innervation and vascularization of DsRed2 fluorescent hindpaw tumors. Pain **115:1-2**, 95-106

Wacnik PW, Eikmeier LJ, Ruggles TR, et al. (2001) Functional interactions between tumor and peripheral nerve: Morphology, algogen identification and behavioral characterization of a new murine model of cancer pain. J Neurosci **21**, 9355-9366

Wacnik PW, Eikmeier LJ, Simone DA, et al. (2005b) Nociceptive characteristics of tumor necrosis factor α in naive and tumor-bearing mice. Neuroscience **132**, 479–491

Wacnik PW, Wilcox GL, Clohisy DR, et al. (2000) Cancer Pain Mechanisms and Animal Models of Cancer Pain. Progr Pain Res Manag **16,** 615-637

Walsh D (2005) Advances in opioid therapy and formulations. Support Care Cancer **13**, 138-144

Wan Y, Wilson SG, Han JS, et al. (2001) The effect of genotype on sensitivity to electroacupuncture analgesia. Pain **91**, 5–13

Wang C, de Pablo P, Chen X, et al. (2008) Acupuncture for pain relief in patients with rheumatoid arthritis: a systematic review. Arthritis Rheum **59:9**, 1249-1256

Wang et al., 1999 Y.Q. Wang, X.D. Cao and G.C. Wu, Role of dopamine receptors and the changes of the tyrosine hydroxylase mRNA in acupuncture analgesia in rats. Acupunct Electrother Res **24**, 81–88

Wang HH, Zhu YH, Xu SF (1994) The potentiation effect of haloperidol on the binding of etorphine to brain membranes in acupuncture analgesia. [Article in Chinese, English abstract]. Acta Physiol Sin **46**, 313–319

Wang K, Yao S, Xian Y, et al. (1985) A study on the receptive field of acupoints and the relationship between characteristics of needling sensation and groups of afferent fibers. Sci Sin (B) **28**, 963–971

Wang Q, Mao LM, Han JS (1990) The arcuate nucleus of hypothalamus mediates low but not high frequency electroacupuncture analgesia in rats. Brain Res **513**, 60–65

Wang Q, Mao LM, Han JS (1991) The role parabrachial nucleus in high frequency electroacupuncture analgesia in rats. Chin J Physiol Sci **7**, 363–371

Wang SM, Kain ZN, White P (2008) Acupuncture analgesia: I. The scientific basis. Anesth Analg **106**, 602–610

Wang Y, Zhang Y, Wang W, et al. (2005) Effects of synchronous or asynchronous electroacupuncture stimulation with low versus high frequency on spinal opioid release and tail flick nociception. Exp Neurol **192**, 156–162

Wang YQ, Cao XD, Li KY, et al. (1997) Relationship between electroacupuncture analgesia and dopamine receptors in nucleus accumbens. Acta Pharmacol Sin **18**, 494–496

Watkins LR, Hutchinson MR, Johnston IN, et al. (2005) Glia: novel counter-regulators of opioid analgesia. Trends Neurosci **28**, 661–669

Watkins LR, Hutchinson MR, Ledeboer A, et al. (2007) Glia as the “bad guys”: implications for improving clinical pain control and the clinical utility of opioids. Brain Behav Immun **21**, 131–146

Watkins LR, Kinscheck IB, Kaufman EFS, et al. (1985) Cholecystokinin antagonists selectively potentiate analgesia induced by endogenous opiates. Brain Res **327**, 181–190

Watkins LR, Mayer DJ (1982) Organization of endogenous opiate and nonopiate pain control systems. Science **216**, 1185-1192

Wax WA, White KR (2000) A short history of a long tradition. Qi: the basis of Chinese medicine. Am J Nurs **100:9**, 42-43

Waxman SG, Cummins TR, Dib-Hajj S, et al. (1999) Sodium channels, excitability of primary sensory neurons, and the molecular basis of pain. Muscle Nerve **22**, 1177–1187

Weber K, Damron TA, Frassica FJ, et al. (2008) Malignant bone tumors. Instr Course Lect **57**, 673-688

Wei JY, Chang SC, Feng CC (1976) Activation of unmyelinated muscle afferents by acupuncture or pressure exerted on muscle. [Article in Chinese, English abstract]. Kexue Tongbao **21**, 505–506

Wei JY, Chang SC, Feng CC (1978) Activation of unmyelinated muscle afferents by acupuncture or pressure exerted on muscle. [Article in Chinese, English abstract]. Acta Zool Sin **24**, 21–28

Wei JY, Feng CC, Chu TH (1973) Observation on activity of deep tissue receptors in cat hindlimb during acupuncture. Kexue Tongbao **18**, 184–186

Wei YF, Liu YL, Zhang SH, et al. (2007) Effect of electroacupuncture on plasma estrin and bone mineral density in ovariectomized rats. [Article in Chinese, English abstract]. Zhen Ci Yan Jiu **32:1**, 38-41

Weissman DE, Dahl JL (1990) Attitudes about cancer pain: a survey of Wisconsin's first-year medical students. J Pain Symptom Manag **5:6**, 345-349

Wells E, Jackson R, Diaz O, et al. (1995) Acupuncture as an adjunct to methadone treatment services. Am J Addict **4**, 198-214

Wen H, Cheung S (1973) Treatment of drug addiction by acupuncture and electric stimulation. Asian J Med **9**, 138-141

White F, Wilson N (2010) Opiate-induced hypernociception and chemokine receptors. Neuropharmacology **58:1**, 35-37

Wick F, Wick N, Wick MC (2007) Morphological analysis of human acupuncture points through immunohistochemistry. Am J Phys Med Rehabil **86**, 7–11

Willis WD, Chung JM (1987) Central mechanisms of pain. JAVMA **191**, 1200-1202

Willis WD, Westlund KN (1997) Neuroanatomy of the Pain System and of the Pathways That Modulate Pain. J Clin Neurophys **14:1**, 2-31

Winkler K, Beron G, Delling G, et al. (1988) Neoadjuvant chemotherapy of osteosarcoma: results of a randomized cooperative trial (COSS-82) with salvage chemotherapy based on histological tumor response. J Clin Oncol **6:2**, 329-337

Withrow SJ, Doige CE (1980) En block resection of a juxtacortical and three intraosseous osteosarcomas of the zygomatic arch in dogs. J Am Anim Hosp Assoc **16**, 867-872

Wittig JC, Bickels J, Priebat D, et al. (2002) Osteosarcoma: A Multidisciplinary Approach to Diagnosis and Treatment. Am Fam Physician **65:1123-32**, 1135-1136

Wolfesberger B, Walter I, Hoelzl C, et al. (2006) Antineoplastic effect of the cyclooxygenase inhibitor meloxicam on canine osteosarcoma cells. Res Vet Sci **80:3,** 308-316

Wong Fl, Boice JD, Abramson DH, et al. (1997) Cancer incidence after retinoblastoma. Radiation dose and sarcoma risk. JAMA **278**, 1262-1267

Wood JN, Boorman JP, Okuse K, et al. (2004) Voltage-gated sodium channels and pain pathways. J Neurobiol **61**, 55–71

Wright B (1987) Clinical pain management techniques in cats. Clin Tech Small Anim Pract **17**, 151-157

Wu CP, Chao CC, Zhao ZQ, et al. (1974) Inhibitory effect produced by stimulation of afferent nerves on responses of cat dorsolateral fasciculus fibers to nocuous stimulus. Sci Sin **XVII**, 688–697

Wu CP, Zhao ZQ, Cheng Y, et al. (1978) Membrane potential changes of dorsal horn neurons elicited by electroacupuncture. Acupunct Anaesthesis **1**, 33–34

Wu G, Jiang JW, Wu GC, et al. (1990) Potentiation of electroacupuncture anagelsia by l-tetrahydropalmatine and its analogues. [Article in Chinese, English abstract]. Acta Pharmacol Sin **11**, 116–119

Wu GC, Zhu J, Cao XD (1995) Involvement of opioid peptides of the preoptic area during electroacupuncture analgesia. Acupunct Electrother Res **20**, 1–6

Wu JN (1996) A short history of acupuncture. J Altern Complement Med **2:1**, 19-21

Wu MT, Hsieh JC, Xiong J, et al. (1999) Central nervous pathway for acupuncture stimulation: localization of processing with functional MR imaging of the brain—preliminary experience. Radiology **212**, 133–141

Wu MT, Sheen JM, Chuang KH, et al. (2002) Neuronal specificity of acupuncture response: a fMRI study with electroacupuncture. Neuroimage **16**, 1028–1037

Xie GX, Han JS, Hollt V (1983) Electroacupuncture analgesia blocked by microinjection of anti-beta-endophin antiserum into periaquedutal grey of the rabbit. Int J Neurosci **18**, 287–292

Xie H (2004) Geriatric Medicine: A Holistic Approach. In: Proceedings of The North American Veterinary Conference: Small Animal Edition Volume 17 Orlando, Florida, January 18 to 22, 65-67

Xie H, Asquith RL, Kivipelto J (1996) A review of the use of acupuncture for treatment of equine back pain. J Equine Vet Sci **7**, 285-290

Xie H, EA Ott, Colahan P (2001) Influence of acupuncture on experimental lameness in horses. In: Proceed Am Assoc Equine Pract, San Diego **47**, 347-357

Xie, H, Colahan P, Ott EA (2005) Evaluation of electroacupuncture treatment of horses with signs of chronic thoracolumbar pain. JAVMA **227:2**, 281-286

Xu GY, Huang LYM, Zhao ZQ (2000) Activation of silent mechanoreceptive cat C and Aδ sensory neurons and their SP expression following peripheral inflammation. J Physiol **528**, 339–348

Xu SF, Li WP, Sheng MP, et al. (1980) The camp level in caudate perfusate during acupuncture analgesia. [Article in Chinese, English abstract]. Acta Physiol Sin **32**, 238–244

Yaksh TL (1984b) Multiple opioid receptor systems in brain and spinal cord: Part 2. Eur J Anaesthesiol **1:3**, 201-243

Yaksh TL(1984a) Multiple opioid receptor systems in brain and spinal cord: Part 1. Eur J Anaesthesiol **1:2**, 171-199

Yaksh TL, Farb DH, Leeman SE (1979) Intrathecal capsaicin deplete substance P in the rat spinal cord and produces prolonged thermal analgesia. Science **206**, 481–483

Yaksh TL, Jessell TM, Games R, et al. (1980) Intrathecal morphine inhibits substance P release from mammalian spinal cord in vivo. Nature **286**, 155–157

Yamamoto J, Kawamata T, Niiyama Y, et al. (2008) Down-regulation of mu opioid receptor expression within distinct subpopulations of dorsal root ganglion neurons in a murine model of bone cancer pain. Neuroscience **151:3**, 843-853

Yan B, Li K, Xu JX, et al. (2005) Acupoint-specific fMRI patterns in human brain, Neurosci Lett **383**, 236–240

Yang J, Lin BC (1992) Hypothalamic paraventricular nucleus plays a role in acupuncture analgesia through the central nervous system in the rat. Acupunct Electrother Res **17**, 209–220

Yang J, Liu WY, Song CY, et al. (2006a) Through central arginine vasopressin, not oxytocin and endogenous opiate peptides, glutamate sodium induces hypothalamic paraventricular nucleus enhancing acupuncture analgesia in the rat. Neurosci Res **54**, 49–56

Yang J, Liu WY, Song CY, et al. (2006b) Only arginine vasopressin, not oxytocin and endogenous opiate peptides, in hypothalamic paraventricular nucleus play a role in acupuncture analgesia in the rat. Brain Res Bull **68**, 453–458

Yang J, Song CY, Lin BC, et al. (1992) Effects of stimulation and cauterization of hypothalamic paraventricular nucleus on acupuncture analgesia. [Article in Chinese, English abstract]. Acta Physiol Sin **44**, 455–460

Yarnold JR (1999) 8 Gy single fraction radiotherapy for the treatment of metastatic skeletal pain: randomised comparison with a multifraction schedule over 12 months of patient follow-up. Bone Pain Trial Working Party. Radiother Oncol **52:2**, 111-121

Ye WL, Feng XC, Chao DF, et al. (1979) Effect of electric stimulation of "Du-Channel" point on 5-HT and 5-HIAA contents in the caudate nucleus in the rabbit. [Article in Chinese, English abstract]. Kexue Tongbao **24**, 235–236

Yin QZ, Mao JR, Guo SY (1988) Changes of reactions of neurons in dorsal raphe nucleus and locus coeruleus to electroacupuncture by hypothalamic arcuate nucleus stimulation. Funct Neurol **3**, 263–273

Yonehara N (2001) Influence of serotonin receptor antagonists on substance P and serotonin release evoked by tooth pulp stimulation with electro-acupuncture in the trigeminal nucleus caudalis of the rabbit. Neurosci Res **40**, 45–51

Yonehara N, T. Sawada T, H. Matsurra H, et al. (1992) Influence of electro-acupuncture on the release of substance P and the potential evoked by tooth pulp stimulation in the trigeminal nucleus caudalis of the rabbit. Neurosci Lett **142**, 53–56

Young FE (1991) Public health report on fluoride benefits and risks. JAMA **266**, 1061-1067

Yu W, Hao JX, Xu XJ, et al. (1998) Long-term alleviation of allodynia-like behaviors by intrathecal implantation of bovine chromaffin cells in rats with spinal cord injury. Pain **74**, 115–122

Yu Y, Kasahara T, Sato T, et al. (1998) Role of endogenous interferon-γ on the enhancement of splenic NK cell activity by electroacupuncture stimulation in mice. J Neuroimmunol **90,** 176-186

Yuan J, Purepong N, Kerr DP, et al. (2008) Effectiveness of acupuncture for low back pain: a systematic review. Spine **33:23**, E887-900

Yuan J, Zhou R (1993) Effect of acupuncture on T-lymphocyte and its subsets from the peripheral blood of patients with malignant neoplasm. [Article in Chinese, English abstract] Zhen Ci Yan Jiu **18:3**, 174-177

Zaslawski CJ, Cobbin D, Lidums E, et al. (2003) The impact of site specificity and needle manipulation on changes to pain pressure threshold following manual acupuncture: a controlled study. Complement Ther Med **11:1**, 11-21

Zhang D, Ding GH, Shen XY, et al. (2007a) Influence of mast cell function on the analgesic effect of acupuncture of "Zusanli" (ST 36) in rats. [Article in Chinese, English abstract]. Acupunct Res **31**, 147–152

Zhang DX, Gu XG, Shan HY (1978) Facilitation and depression exerted by the discrete loci of the head of the caudate nucleus in rabbits. [Article in Chinese, English abstract]. Acta Physiol Sin **30**, 21–28

Zhang DX, Gu XG, Shan HY (1980) Modification of pain reaction by electrical stimulation of different loci of the head of caudate nucleus in rabbits. [Article in Chinese, English abstract]. Acta Physiol Sin **32**, 159–165

Zhang GG, Yu C, Lee W, et al. (2005a) Involvement of peripheral opioid mechanisms in electroacupuncture analgesia. EXPLORE: J Sci Healing **1**, 365–371

Zhang H, Cang CL, Kawasaki Y, et al. (2007b) Neurokinin-1 receptor enhances TRPV1 activity in primary sensory neurons via PKC: a novel pathway for heat hyperalgesia. J Neurosci **27**, 12067–12077

Zhang J, Ng D, Sau A. J, Effects of electrical stimulation of acupuncture points on blood pressure. Chiropr Med **8:1**, 9-14

Zhang L, Zhang YQ, Zhao ZQ (2005b) Anterior cingulate cortex contributes to the descending facilitatory modulation of pain via dorsal reticular nucleus. Eur J Neurosci **22**, 1141–1148

Zhang LX, Li XL, Wang L, et al. (1997) Rats with decreased brain cholecystokinin levels show increased responsiveness to peripheral electrical stimulation-induced analgesia. Brain Res **745**,158-164

Zhang RX, Lao L, Wang LB, et al. (2004a) Involvement of opioid receptors in electroacupuncture-produced anti-hyperalgesia in rats with peripheral inflammation. Brain Res **1020**, 12–17

Zhang RX, Lao L, Wang X, et al. (2004b) Electroacupuncture combined with indomethacin enhances antihyperalgesia in inflammatory rats. Pharmacol Biochem Behav **78**, 793–797

Zhang RX, Li A, Liu B, et al. (2007c) Electroacupuncture attenuates bone cancer pain and inhibits spinal interleukin-1 beta expression in a rat model. Anesth Analg **105**, 1482–1488

Zhang RX, Li A, Liu B, et al. (2008) Electroacupuncture attenuates bone-cancer-induced hyperalgesia and inhibits spinal preprodynorphin expression in a rat model. Eur J Pain **12:7**, 870-878

Zhang RX, Liu B, Wang L, et al. (2005c) Spinal glial activation in a new rat model of bone cancer pain produced by prostate cancer cell inoculation of the tibia. Pain **118**, 125–136

Zhang RX, Wang L, Wang X, et al. (2005d) Electroacupuncture combined with MK-801 prolongs anti-hyperalgesia in rats with peripheral inflammation. Pharmacol Biochem Behav **81**, 146–151

Zhang WT, Jin Z, Cui GH, et al. (2003a) Relations between brain network activation and analgesic effect induced by low versus high frequency electrical acupoint stimulation in different subjects: a functional magnetic resonance imaging study. Brain Res **982**, 168–178

Zhang WT, Jin Z, Luo F, et al. (2004) Evidence from brain imaging with fMRI supporting functional specificity of acupoints in human. Neurosci Lett **254**, 50–53

Zhang WT, Z. Jin Z, J. Huang J, et al. (2003b) Modulation of cold pain in human brain by electric acupoint stimulation: evidence from fMRI. NeuroReport **14**, 1591–1596

Zhang X, Wenk HN, Gokin AP, et al. (1999) Physiological studies of spinohypothalamic tract neurons in the lumbar enlargement of monkeys. J Neurophysiol **82:2**, 1054-1058

Zhang YW, Zhang NN, Qian H (1989) Influence of electroacupuncture on ACh content and AChE activity in the spinal ganglia of rat. [Article in Chinese, English abstract]. Zhen Ci Yan Jiu **14:4**, 463-7

Zhao ZQ (2008) Neural mechanism underlying acupuncture analgesia. Prog Neurobiol **85**, 355-375

Zhao ZQ, Duggan AW (1988) Idazoxan blocks the action of noradrenaline but not spinal inhibition from electrical stimulation of the locus coeruleus and nucleus Kolliker-Fuse of the cat. Neuroscience **25**, 997–1005

Zheng L, Li X, Li H, et al. (1995) Effect of brain somatostatin on electroacupuncture analgesia of rat. [Article in Chinese, English abstract]. Acupunct Res **20**, 22–25

Zhou PH, Qian PD, Huang DK, et al. (1979) A study of the relationships between the points of the channels and peripheral nerves. National Symposia of Acupuncture-Moxibustion & Acupuncture Anesthesia Beijing, 302

Zhou Y, Sun YH, Shen JM, et al. (1993) Increased release of immunoreactive CCK-8 by electroacupuncture and enhancement of electroacupuncture analgesia by CCK-B antagonist in rat spinal cord. Neuropeptides **24**, 139–144

Zhou ZF, Jin WQ, Han JS (1984) Potentiation of electroacupuncture analgesia and morphine analgesia by intraventricular injection of thiophan and bestatin in the rabbit [Article in Chinese, English abstract]. Acta Physiol Sin **36**, 175–182

Zhou ZQ (2008) Neural mechanism underlying acupuncture analgesia. Prog Neurobiology **85:4**, 355-375

Zhu B, Xu WD, Rong PJ, et al. (2004a) A C-fiber reflex inhibition induced by electroacupuncture with different intensities applied at homotopic and heterotopic acupoints in rats selectively destructive effects on myelinated and unmyelinated afferent fibers. Brain Res **1011**, 228–237

Zhu CB, Li XY, Zhu YH, et al. (1995) Binding sites of mu receptor increased when acupuncture analgesia was enhanced by droperidol: an autoradiographic study. Acta Pharmacol Sin **16**, 311–314

Zhu CB, Li XY, Zhu YH, et al. (1997b) Alteration of monoamine contents in microdialysate following droperidol enhanced electroacupuncture [Article in Chinese, English abstract]. Acta Physiol Sin **49**, 382–388

Zhu CB, Xu SF, Wu GC, et al. (1997a) Research on combination of acupuncture with drugs. World J Acupunct Moxib **7**, 54–59

Zhu LX, Li C, Ji C, et al. (1990a) Involvement of GABA in acupuncture-induced segmental inhibition. Eur J Pain **11**, 114–118

Zhu LX, Li CY, Yang B, et al. (1990b) The effect of neonatal capsaicin on acupuncture analgesia [Article in Chinese, English abstract]. Acupunct Res **15**, 285–291

Zhu LX, Ye Y, Mo XR, et al. (2002) The important role of activation of GABAb receptors in acupuncture analgesia [Article in Chinese, English abstract]. Acupunct Res **27**, 85–91

Zhu LX, Zhao FY, Cui RL (1991) Effect of acupuncture on release of substance P. Ann NY Acad Sci **632**, 488–489