Acupuncture Analgesia for Animals

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Yi-Wen Lin and Jaung-Geng Lin

Abstract

Acupuncture is part of Chinese traditional medicine (TCM), and is an ancient system of healing that is well documented for its therapeutic effects in pain management. In ancient Chinese medical text, acupuncture is described as the primary treatment for several clinical problems, especially in pain management. In early times, acupuncture was practiced with primitive sharp stones or bamboo that have been replaced over time with ultra-fine needles. Acupuncture is the art of inserting fine needles into specific points on the skin, called acupoints, to initiate a phenomenon such as *de-qi*. *De-qi* is a crucial indicator during acupuncture treatments, marked by some obvious feelings such as soreness, heaviness, fullness, numbness, migration. There are over 360 acupoints located along 14 main body meridians traversing the head, arms, legs and trunk. All meridians are symmetrical, traversing both sides of the body, except for the single meridians known as the Conception and Governor Vessels, which run along the body's midline. In general, it is difficult to associate meridians with any anatomical or physiological structures. However, virtually all acupoints are located in deep tissues with abun-

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dant sensory nerve terminals, suggesting that a strong relationship exists between the meridian points and viscera in relation to nerve connection. It is thought that peripheral A and C fibers may mediate the afferent transmission of the acupuncture signals and ameliorate painful sensation. Recently, the World Health Organization (WHO) reported on the efficacy and safety of acupuncture treatment in over 30 effective symptoms, conditions and diseases. Acupuncture is most often used for pain relief, although it is also used for many other conditions.

Keywords
Acupuncture · Chinese medicine · Inflammatory pain · Fibromyalgia pain · Neuropathic pain

2.1 Introduction

The effectiveness of acupuncture in the relief of pain was first reported in the 1970s by Johnson and colleagues (Johnson 1973). In ancient times, acupuncturists used manual manipulation methods to achieve clinical benefits. Today, electroacupuncture has become more popular, especially in pain management. Recently, several studies have suggested that acupuncture increases the release of endogenous opiates (Han 2003), serotonin (Chang et al. 2004) and adenosine (Goldman et al. 2010) and thereby produces its anti-nociceptive effect. The major mechanism underlying acupuncture analgesia is the release of opiates in the CNS. Transcutaneous electric stimulation of acupoints can reduce opioid intake and opioid adverse effects after intraabdominal surgery (Wang et al. 1997). Pretreatment with EA can reliably reduce postoperative analgesic requirements and side effects of analgesics in patients undergoing lower abdominal surgery (Lin et al. 2002). Manual acupuncture can increase pain threshold accompanied by releasing morphine-like factor and further blocked by opioid receptor antagonist naloxone (Han 2003). Chen and Han indicate that EA analgesia at 2 Hz is elicited in rats by activating μ opioid receptors (Chen and Han 1992). In contrast, EA at 100 Hz can release dynorphins that bind to κ opioid receptors in the spinal cord of rats (Chen and Han 1992). Moreover, injection of κ opioid receptor antagonist reliably attenuates analgesia elicited by EA 2 Hz, whereas the analgesic effect of EA 100 Hz is alleviated by κ-opioid receptor antagonist treatment. Subsequently, mixed-frequency stimulation (2 and 100 Hz) has been used to reduce acupuncture tolerance and increase the curative effect over that produced by single-frequency stimulation (Hamza et al. 1999). Ankle joint mobilization has been found to attenuate postoperative pain by activating the peripheral opioid system (Martins et al. 2012). Opiate receptors not only exist in the brain and spinal cord but also in peripheral sensory neurons (Stein 2003). Paradoxically, chronic opiate administration in humans has been

associated with hyperalgesia associated with hyperexcitability and functional remodeling of tetrodotoxin-resistant (TTX-R) Na^+ and transient receptor potential vanilloid 1 (TRPV1) channels of sensory neurons (Ross et al. 2012). In clinical medicine, opiates are the most commonly used and most powerful analgesics available for severe acute and chronic pain. Opiate use is associated with a significant risk of systemic side effects including addiction, tolerance, respiratory depression, nausea, and more. As mentioned earlier, a novel and curative generation of opiates that target the peripheral site, is emerging. Opiates that have a peripheral site of action in inflamed tissue selectively activate peripheral opioid receptors and avoid centrally-mediated side effects. Acupuncture has important advantages over opiates in the treatment of pain, including convenience, low costs, curative ability, and a lower risk of side effects.

Acupuncture has been reported to increase the release of adenosine at peripheral sites (Goldman et al. 2010). Needling can trigger a widespread increase in purines including adenosine and adenosine 5′-triphosphate (ATP), which is consistent with an elevation in adenosine levels after tissue damage. The anti-nociceptive effects of adenosine A1 receptors in both peripheral and central regions are well documented, but these agents are associated with dramatic site effects, especially in relation to the heart. Adenosine A1 receptor agonists reportedly reduce inflammatory and neurogenic pain. Evidence suggests that acupuncture reliably increases both ATP and adenosine at peripheral sites and thereby attenuates inflammatory and neuropathic pain (Goldman et al. 2010). These mechanisms indicate that the adenosine A1 receptor (A1R) is crucial for acupuncture analgesia. Prostatic acid phosphatase (PAP) is expressed in nociceptive neurons and functions as an ectonucleotidase to increase adenosine concentrations adenosine at the peripheral level. PAP reportedly exists in skeletal muscle located near the Zusanli acupoint, and serves as a rate-limiting ectonucleotidase that increases adenosine concentrations (Goldman et al. 2010; Quintero et al. 2007). As a result of this activation, painful peripheral neuropathy is eliminated in mice. Intravenous injection of adenosine can usually relieve postoperative pain and reduce opiate use (Gan and Habib 2007). The antinociceptive effects of PAP were evaluated in preclinical investigations to determine whether PAP sustains A1R activation followed by depletion of phosphatidylinositol 4,5-bisphosphate (PIP2) (Gan and Habib 2007). Intrathecal injection of human PAP (hPAP) can induce A1R-dependent antinociception in preclinical models of inflammatory pain and neuropathic pain (Sowa et al. 2010). The increase in adenosine levels induced by acupuncture has a short antinociceptive effect, peaking at 30 min (Goldman et al. 2010). We urgently need to find a way of achieving prolonged biologic effects from acupuncture. Some researchers have suggested that injection of hPAP at BL40 acupoints provides a novel way of generating longer-lasting antinociception (Hurt and Zylka 2012).

The clinical use of acupuncture is associated with several advantages such as convenience, low cost, and fewer side effects, compared with pharmacologic therapy. In particular, acupuncture has demonstrated high efficacy in the relief

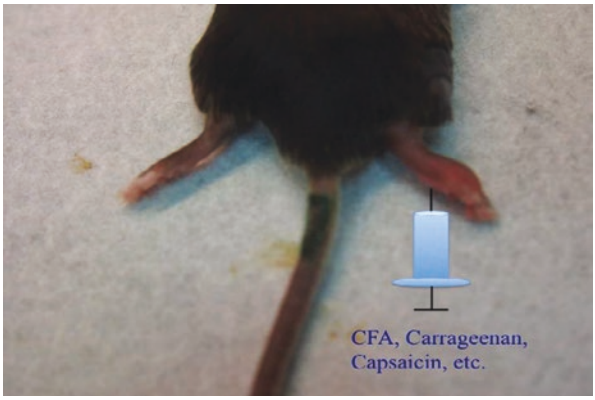
of pain-related symptoms. Electroacupuncture (EA) has demonstrated improvements in several spinal cord injury (SCI) parameters: neurologic recovery scores; application of high-frequency EA at 75 Hz to the BL-62 and SI-3 acupoints limits SCI-related damage to spinal cord neurons and axons (Wong et al. 2003); and enhances recovery of bladder function in acute SCI (Liu et al. 2013). In a small study, manual acupuncture at the BL-33 acupoint reduced urinary incontinence in 15% of 13 patients with chronic SCI and improved incontinence by at least 50% in another 46% (Honjo et al. 2000). Almost all patients with SCI will suffer from pain symptoms, especially neuropathic and musculoskeletal pain (Cardenas and Jensen 2006; Widerstrom-Noga et al. 2001). The evidence in these studies suggests the effectiveness of acupuncture for SCI and its complications. Animal experimental models suggest that several potential mechanisms explain the beneficial clinical effects of acupuncture, including a reduction in glial fibrillary acidic protein (GFAP) in the injured cord, thereby inhibiting reactive astrocyte proliferation and reducing glial scar formation (Politis and Korchinski 1990), and a reduction in epidermal growth factor receptor (EGFR) levels, suggesting less scar formation Politis and Korchinski 1990. EA also reduces free radical formation, and down-regulates AQP-4 (aquaporin) expression after SCI, thereby inhibiting spinal cord edema that can produce secondary spinal cord damage. In addition, acute SCI models in animals have shown that EA reduces spinal cord atrophy, with a two-third reduction of anterior horn neuron loss, and reduces the acute stress response as measured by serum cortisol levels (Politis and Korchinski 1990). EA has also shown the potential to enhance spinal cord regeneration, with earlier and higher levels of laminin expression in the injured cord in EA-treated animals (Zhu 2002). Notably, Wu et al. reported that acupuncture reverses elevations in acetylcholinesterase and succinate dehydrogenase and reductions of acid phosphatase in the anterior horn of the spinal cord observed in experimental SCI, which could inhibit or delay the deterioration of those anterior horn cell neurons (Wu et al. 1999). High-frequency EA causes release of dynorphins (Han 2003). In SCI patients with moderate to severe pain, acupuncture improved pain intensity and SCI-related sequelae (Nayak et al. 2001).

Rheumatoid arthritis (RA) treatment includes disease-modifying antirheumatic drugs (DMARDs), which have proven to be beneficial in the treatment of RA. However, DMARDs can have a wide range of side effects, depending on the drug used, such as suppression of the immune system, leading to an increased risk of side effect (Galarza-Delgado et al. 2017; Nam et al. 2017). Acupuncture avoids these side effects and can effectively relieve RA-elicited symptoms and enhance the patient's quality of life, especially in regard to pain management. Acupuncture is noticed to expand joint motion and improve emotion by modulating of immune system, nerve system, endocrine system, etc. The adjuvant uses of auricular EA resulted in significant short- and long-term treatment effects in the treatment of patients with RA (Bernateck et al. 2008).

2.2 Inflammatory Pain

Inflammatory pain is disabling, and difficult to treat clinically. A number of animal models reproduce human inflammatory processes, such as the model of carrageenan-induced paw edema, via complete Freund's adjuvant (CFA)-induced paw edema, capsaicin, collagen-induced arthritis (CIA), amongst others. Inflammatory pain is often associated with peripheral tissue damage, ischemia, hypoxia, acidosis, and aggregation of inflammatory mediators that can further increase pain sensitivity and lower the pain threshold (Steen et al. 1996; Walder et al. 2010). Damaged tissue also releases endogenous inflammatory factors that activate nociceptive fibers or nearby non-neural cells (mast cells, macrophages, platelets, and immune cells). Nerve terminals are induced by the inflammatory mediators to deliver painful signals (Julius and Basbaum 2001).

CFA and carrageenan are most often used to initiate a mice inflammatory pain by injecting into the peripheral site for inducing either cell immune or non-cell immune inflammatory pain (Ikeuchi et al. 2009; Chen et al. 2011; Huang et al. 2013). Increased painful response was induced successfully and can be further divided into two pain subtypes. Painful sensation to harmful stimuli at original injection site is defined as primary hyperalgesia. For example, inflammatory pain sensation can be induced with carrageenan injected into the origin knee joint (Ikeuchi et al. 2009). In contrast, inflammatory mediators induced hyperalgesia to noxious stimuli maybe also induced outside of the injection site and often considered as secondary hyperalgesia. With this issue, both mechanical and thermal hyperalgesia of the hindpaw was induced in mice after carrageenan inflammation in the muscle (Sluka et al. 2007). Both acute and chronic inflammatory pain can be induced and further maintained for 30 days. Pain is not only a single phenomenon but can be separated into different directions. Accordingly, emotional dimension from pain induction can also been associated with the feelings of unpleasantness that can infect unpleasant emotions secondary effects (Price et al. 2001). Pain can be further separated to at least four categories: nociceptive, inflammatory, neuropathic and idiopathic pain. Nociceptive and inflammatory pains are often acute and short-term effect. In contrast, neuropathic and idiopathic pains can be maintained for at least for several months to years. Inflammatory pain models can now also be initiated for a longer time. Investigator can induce different duration of inflammatory pain according to which mediators they injected. Formalin test can be used to induce both inflammatory and non-inflammatory pain at different phase (Hunskaar and Hole 1987), and injection of carrageenan or CFA into the peripheral tissue to induce inflammatory pain. Neuropathic pain is more complicated with multiple etiological factors that caused by damage to nerve fibers by different manipulation. The manipulation can further affect the somatosensory system with a long-term effect. Bennett and colleagues have developed a partial nerve injury animal model by loose ligation of the nerve for the first time (Bennett and Xie 1988). Accordingly, several surgical strategies have been used by using cuts, ligations, freezing to induce neuropathic pain (Shields et al. 2003).



Induction of inflammatory pain by injection of CFA, carrageenan or capsaicin into hindpaw

In acupuncture research, CFA injection is the animal model most commonly used to induce peripheral inflammatory pain (Chen et al. 2011; Huang et al. 2013). Advantages of the CFA model include its high rate of success in the induction of inflammatory pain, convenience, and low cost. The acupoints that are selected for inflammatory pain relief are GB30 and ST36 (as shown in Table 2.1, permit no. 2017-061). Accurately locating those acupoints that are usually used in mice or rats is crucial for EA research. As shown in Fig. 2.1, GB30, GB34, ST36, SP6, and BL60 are usually selected for pain relief as according to advice given in the human's atlas and ancient Chinese medicine.

2.3 Potential Mechanisms Underlying the Effects of Acupuncture in Inflammatory Pain

Recent articles have suggested that EA can reduce pain through a mechanism that reduces phosphorylation of N-Methyl-D-aspartate (NMDA) receptor (Ryu et al. 2008). Other researchers have found that 2 Hz EA at the Zusanli and Sanyinjiao acupoints reduces thermal hypersensitivity of the hind paw induced by CFA injection (Jang et al. 2011). The same result can be achieved by injecting dizocilpine, an NMDAR antagonist. The antinociceptive effects of these single agents may be increased by the combined administration of EA and dizocilpine. Notably, phosphorylation of ERK is increased during the inflammatory process and can be decreased by both dizocilpine and EA delivered to the spinal cord (Jang et al. 2011).

Central sensitization of nociceptive transmission may be initiated in inflammatory, neuropathic, and postoperative pain syndromes, and NMDAR activation may further increase Ca^{2+} influx and activation of second messenger pathways underlying painful sensations. Jung et al. reported that intracellular Ca^{2+} is a crucial target in analgesia associated with 2 Hz EA, and can be modulated through spinal NMDAR (Jung et al. 2010). NMDARs can also be regulated by several protein kinases and phosphatases, and it has been suggested that protein phosphatases 1 and 2A have a crucial role in EA-mediated analgesia through the regulation NMDA receptor phosphorylation in the spinal cord (Ryu et al. 2008).

Table 2.1 Acupoints GB30 and ST36 are most often used for EA manipulation

Injection	Type	Frequency	Amplitude	Acupoints	Behavior	Source		
CFA	EA	10 100	3 mA	GB30	T			Zhang et al. (2005)
		10	3 mA	GB30	T		Zhang et al. (2005)	
		10	3 mA	GB30	PPC			
		30	2 mA	GB30	T	Zhang et al. (2005)		
		60 4	1	GB30-34	T			
		2 100	1	GB30-34	M and T			
		2 100	1	GB30-34				
		60 2	<1	GB30-34	T			
		4 16	0.5–1–1.5 V	GB30-34	T	Wang et al. (2006)		
		2 100 alt	1 2	ST36 BL60	M			
		100-4 alt	1	ST36 BL60	T			
		2	1	ST36 SP6	T	Jang et al. (2011)		
		1 15 100		ST36 SP6	M			
		100	0.5–1–1.5 mA	ST36 SP6	M and T			
		100	1 1.2 1.5	ST36 SP6	TF			
	MA			ST36	T			
				ST36	M and T	Goldman et al. (2010)	*PPC* pain-paired compartment	
				ST36	FF		*TF* tail flick	

(continued)

Table 2.1 (continued)

Carrageenan	EA	10	1	ST36 SP6	M		*M* mechanical	
		2	0.5 1 3	ST36 SP6	T		*T* thermal	
		4	0.6–0.8	ST36 SP6			*FF* flinching frequency	
		2 15 120	1 2 3	ST36 SP6	T			
		3	1–3 mA	ST36	M			
		1 120	1 3	ST36	T			
		100	2	ST36 SP9	M			
		60 2	1 2 3	ST36 BL60	T			
		10	1.2 3	GB30	T			
				SP6	M			

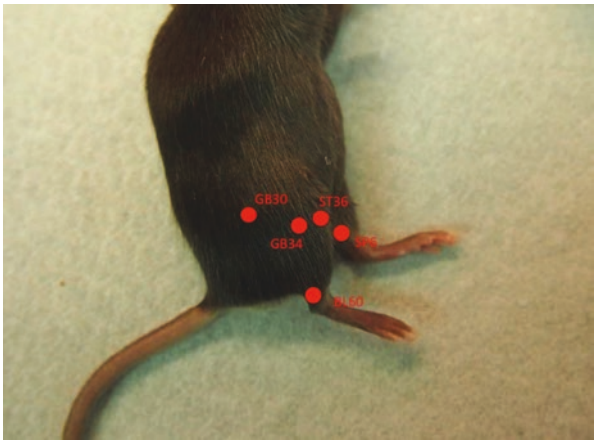


Fig. 2.1 EA manipulation with steel needles inserted into the ST36 and ST37 acupoints

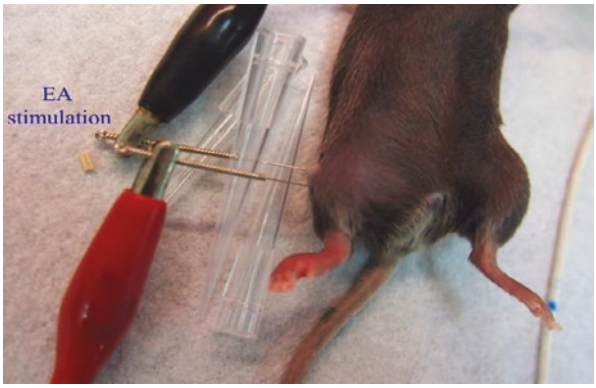


Fig. 2.2 Pharmacological injection of drugs into acupoint

Wang et al. used immunohistochemistry to demonstrate that DRG neurons expressing both IB4 and NR1 are dramatically increased after CFA-mediated inflammatory pain. This increase can be reversed by EA treatment, suggesting a specific role of NMDARs in IB4-positive nociceptive neurons (Wang et al. 2006). Furthermore, medium-to-high frequency EA (10 and 100 Hz) reliably reduced CFA-initiated inflammatory pain, and when EA was used simultaneously with a sub-effective dose of MK-801, the antinociceptive effect was prolonged (Zhang et al. 2005). Improved knowledge about the mechanisms of pain signaling has resulted in the technique of acupoint pharmacological injection, whereby pharmacological agents may be injected directly into acupoints for increased pain relief (Fig. 2.2).

2.4 Fibromyalgia Pain

Fibromyalgia (FM) is a puzzling muscle pain syndrome with chronic widespread and mechanical pain. The prevalence of this debilitating condition is approximated to be 2–8% of the population (five million adults in the U.S.) (English 2014; Clauw

2014). FM is characterized by pain that is spread throughout the body, accompanied by fatigue, depression, memory problems, anxiety, sleep disturbances, and headaches. Chronic symptoms may affect the whole body, and the underlying disease mechanisms are unclear. Research indicates that FM results from an imbalance of several neurotransmitters in the central nervous system, including serotonin, dopamine, and norepinephrine (Riva et al. 2012; Valim et al. 2013). Decreased levels of serotonin have been reported in the cerebrospinal fluid of FM patients, which suggests that central inhibition of pain is reduced (Dadabhoy et al. 2008). Mental traumatic syndromes reduced hypothalamic-pituitary-adrenal (HPA) function, and long-lasting stress have also been associated with FM (Parker et al. 2001). Both peripheral nerve activation and central sensitization may play crucial roles in the development of FM, a chronic pain syndrome that is defined by widespread pain for more than 3 months and the presence of more than 11 trigger points in 18 specialized points (Gerwin 2011). Given the lack of understanding of the disease etiology and the multitude of possible mechanisms, the treatment of FM is complex and many controversial approaches have been adopted, such as drug therapy, exercise, and dietary changes. Currently, there is no cure for FM, but clinicians usually prescribe supporting therapy to ease pain symptoms and improve quality of life. Such treatment includes muscle relaxants, analgesics, antidepressants, and sedatives. Three medications commonly used in clinics for the treatment of FM are supported by the U.S. Food and Drug Administration (FDA): (1) pregabalin, a presynaptic voltage-gated calcium channel blocker that reduces synaptic transmission and thereby attenuates FM-related pain and sleep disturbances (Roth et al. 2014; Smith and Moore 2012); duloxetine, another agent that can ameliorate some FM symptoms, but is not universally effective and is associated with serious side effects (Häuser et al. 2014); and milnacipran, a serotonin-norepinephrine reuptake inhibitor (SNRI) that was designed to treat FM has had moderate success but is accompanied by adverse events, most commonly nausea (Trugman et al. 2014).

The development of treatment for FM has been limited and understanding remains poor as to the mechanisms underlying persistent pain signaling (Vierck Jr. 2006; DeSantana and Sluka 2008). Many animal studies have used a model of FM induced by repeated acidic saline injections into the gastrocnemius muscle (GM). This chronic muscle pain model produces symptoms similar to those experienced in FM patients, with long-lasting, spreading mechanical hyperalgesia, fatigue, sympathetic predominance, and altered central sensitization (Sluka et al. 2001; Pratt et al. 2013; DeSantana et al. 2013). These FM animals are sensitive to antidepressants and anticonvulsants, but not to non-steroidal anti-inflammatory drugs (NSAIDS) (DeSantana and Sluka 2008). FM may result from activation of acid sensing ion channels (ASICs), vanilloid receptor 1 (TRPV1), or others. ASIC3 and TRPV1 are voltage-insensitive cationic channels that are gated by extracellular protons. Interestingly, decreases in tissue pH have been observed in animals with FM, ischemia, arthritis, tumors, or brain trauma, and protons have been shown to activate the terminals of nociceptors *in situ*. Recordings of peripheral dorsal root ganglion (DRG) neurons have shown that extracellular acidification induces inward currents with differing kinetics, ion selectivity, and pH dependence. Although much is

known about how ion channels give rise to peripheral sensation and central sensitization leading to FM pain, less is known about the mechanisms involved in FM pain signaling.

Several different physiological mechanisms may contribute to FM pain. NMDA receptors, ASIC3, TRPV1, calcium channels (Cav), and substance P (SP) have all been implicated as having roles (Sluka et al. 2003; Chen et al. 2010, 2014; Chen and Chen 2014). Dual acid saline injections activate the cAMP pathway in the spinal cord (Hoeger-Bement and Sluka 2003). Activation of ERK, a member of the MAPK family, has also been reported in the anterior paraventricular nucleus of the thalamus (Chen et al. 2010). Administration of neurotrophin-3 reduces acid-induced chronic muscle pain (Gandhi et al. 2004), while pregabalin and the M-type voltage gated potassium channel activator flupirtine have also proven effective in treating muscle pain (Yokoyama et al. 2007; Nielsen et al. 2004). The development and maintenance of this type of chronic muscle hyperalgesia is associated with changes in the amygdala and thalamus (Cheng et al. 2011; Chen et al. 2010). However, information about the detailed mechanisms underlying FM remains very limited.

Controversy surrounds the efficacy and safety of opioids for treating FM pain syndromes. Much evidence suggests that opioids offer little help to FM patients suffering from widespread pain, but some FM patients feel relief following opioid administration. In contrast, in an animal model, FM can be reduced by administering μ- or δ-opioid receptor agonists (Sluka et al. 2002), or glutamate receptor antagonists at the spinal cord level (Skyba et al. 2002). However, opioid use is not ideal in humans, due to the potential for tolerance and addiction. Acupuncture may be advantageous in treating complicated FM syndromes, as it has the ability to reduce pain, anxiety, depression, and sleep disturbance (Chen et al. 2011, 2012; Sniezek and Siddiqui 2013; Li et al. 2014; Mao et al. 2014).

2.5 Possible Mechanisms Underlying FM in Mice

Moderate-intensity aerobic exercise successfully attenuates mechanical hyperalgesia induced in mice by acidic saline injection and increases neurotrophin-3 (NT-3) synthesis in skeletal muscle, and thus is an ideal model for FM. The increase of NT-3 is similar to that observed in humans (Sharma et al. 2010). These researchers also describe a significantly greater increase in NT-3 protein in the GM compared with soleus muscle, which indicates that exercise can concisely induce NT-3 synthesis in a specific muscle target (Sharma et al. 2010). Other researchers have indicated that low-intensity exercise alleviates mechanical hyperalgesia in the FM model through activation of the endogenous opioid system (Bement and Sluka 2005). This aligns with clinical phenomena showing a reduction in FM following low-intensity exercise or morphine injection (Hoeger-Bement and Sluka 2003). Furthermore, a recent study demonstrated that injecting acidic saline (pH 4.0) into the GM in rats induces a bilateral mechanical hyperalgesia similar to that observed with FM in humans (ref). These researchers used

EA at 15 or 100 Hz for 20 min on 5 consecutive days at the ST36 and SP6 acupoints. They suggest that there was a significant reduction of mechanical hyperalgesia in response to EA at 15 or 100 Hz.

2.6 Neuropathic Pain

Neuropathic pain is a physiological and pathological process of the somatosensory system that may originates from either the peripheral or central nervous system (Magrinelli et al. 2013). This pain state is characterized by a persistent stressor that can induce biological, physiological, and pathological alterations that further initiate multiple neuropsychiatric disorders. Neuropathic pain can be very difficult to treat, with around 40–60% of patients achieving only partial relief. Moreover, with a prevalence rate of around 6.9–10% and associated high insurance costs, this disorder is a legitimate health concern (van Hecke et al. 2014). Sodium channel blockers, including carbamazepine and lamotrigine, are often used to relieve neuropathic pain such as trigeminal neuropathic pain (Di Stefano et al. 2014; Lauria et al. 2012). In animal studies, sciatic nerve ligation (SNL), sciatic nerve incision (SNI), and chronic constriction injury (CCI) are usually used in neuropathic pain studies (Fig. 2.3). Several different drug classes are used in the clinical situation, including antidepressants, opiates, and GABAergic drugs, all of which are accompanied by several side effects. Acupuncture offers an alternative therapy that is convenient, cheap, and with few side effects.

The threshold for LTP induction in C-fibers in spinal cord dorsal horn is lower in a rat model of neuropathic pain, and the amplitude of field potentials is higher in these rats (Xing et al. 2007). Interestingly, 2 Hz EA delivered at the ST 36 and SP 6 acupoints can reliably reduce neuropathic pain by inducing LTD in C-fibers (Xing et al. 2007), and the analgesic effect is increased by administration of the NMDAR antagonist MK-801 or the opioid receptor antagonist naloxone. Li and colleagues reported that EA has antidepressive and anxiolytic effects in the neuropathic pain model in rats, accompanied by restoration of NR1 phosphorylation in the

Fig. 2.3 Acupoints that are always used in animal EA experiments

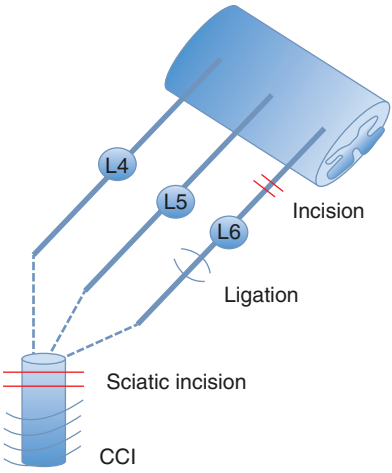


Fig. 2.4 Methods to induce neuropathic pain

hippocampus (Li et al. 2014). Another study demonstrated that 2 and 15 Hz EA reduces CCI-induced neuropathic pain, accompanied by reductions in TRPV4 levels in the cortex instead of the spinal cord (Hsu et al. 2014). Low-frequency EA attenuates cold hypersensitivity (allodynia) in rats that is mediated by the endogenous opioid, but not noradrenergic, system (Moon et al. 2014). The antinociceptive effect of EA is partly mediated by inhibiting glial cell proliferation (Gim et al. 2011). EA can reduce mechanical and thermal hyperalgesia by simultaneously attenuating the expression of P2X3 receptors in DRG neurons from rats suffering from CCI induction. Evidence has also demonstrated that the analgesic effect of EA on chronic neuropathic pain is mediated by P2X3 receptors in rat dorsal ganglion neurons (Tu et al. 2012). The methodology of neuropathic pain is shown in Fig. 2.4. The most commonly used models for inducing neuropathic pain include sciatic nerve incision, ligation, and CCI. The CCI model reliably induces neuropathic pain by setting 4 loose ligatures around the sciatic nerve. The rats were administered isoflurane anesthesia and the sciatic nerve was exposed. A four 4–0 chromic gut sutures were tied loosely to the sciatic nerve to induce CCI model.

2.7 Animal Behavior for Pain Investigation

Animal behavior analysis is used to evaluate painful responses in animals. The von Frey filaments test and mechanical devices are used for measuring mechanical pain thresholds, the plantar thermal sensitivity (Hargreaves) test, tail-flick test, and the hot/cold plate for evaluating thermal analgesia (Mogil 2009; Langford et al. 2010), locomotor activity testing for fatigue, the open-field and forced-swimming tests for detecting depression.

Mechanical hyperalgesia of allodynia can be measured by testing the number of responses to stimulation with 3–5 applications of von Frey filaments. Furthermore, the electronic von Frey test can be used to record withdrawal force units in grams.

Mice were put on a wire mesh platform fixed in a plexi-glass chamber and allowed at least 30 min acclimatization. A von Frey filament of 0.02 g for mice bending force would be used as a basal stimulation. Fine filaments were briefly applied 3–5 times to each hind paw, average as pain threshold, with a 30-s interval between each application. The responses were then considered valid with an abrupt foot lift on application of the fine filament.

For thermal Hargraves' test, the experimental mice were moved to a small chamber on a glass plate and calm down for more than 30 min. The accrue radiant heat source was deliver directly to the hindpaw surface of mice through the clear glass and till the mouse significantly withdrew the hindpaw. The withdraw latency of hindpaw from the beginning of stimulation could be measured by using IITC analgesiometer. The radiant light intensity was set according to breed of mice to obtain a baseline response with a time approximately 10 s. To avoid harmful injury, we should turn down to machine within 30 s to minimize heat damage to the mice skin. Thermal hyperalgesia was measured 3–5 trials for average. Between trials a 10 min recovery period should be held. The thermal responses were measured and defined as the mean from 3 to 5 trials at each measure time point.

Alternatively, both hot and cold plate can be used for high and low thermal induced pain behavior. After induction of pain, animals were placed and measured parameters using a hot/cold plate (IITC Life Sciences, Woodland Hills, CA, USA). Five minutes of animal behavior were recorded by using a digital camera and were further analyzed offline using a personal computer. For hot and cold plate, we can analyze the duration of forepaw licking, the number of jumping instances, the latency for first jump, paw withdraw latency, paw withdraw number, rearing time and number. The tail flick test is often used in pain research, which is similar to hot plate test, by recording the responses to ho on the mice tail. The light beam is located and focused on the mice's tail and record the tail withdraw latency. When the mice feel the high thermal and flick its tail, the time was recorded as its pain threshold. All pain experiments were tested prior to different kind of pain induction at different timing following injections. All tests should be performed at constant room temperature, and all stimuli should be applied since the mice were calm but not sleeping or grooming.

Conclusions

In summary, based on introduction from this section, we conclude that Acupuncture is effective in treating pain syndrome including inflammatory, fibromyalgia, and neuropathic pain. Here we collective most therapeutic effect acupoints for treatment of different pain. We also introduce several approaches to induce animal pain model such as inflammatory, fibromyalgia, and neuropathic pain. This section further describes the detail mechanisms underlying these pain syndromes. This knowledge will further helpful for doctors and researchers to address pain issue for drug development, acupuncture science, and novel technique investigations.

References

Bement MK, Sluka KA. Low-intensity exercise reverses chronic muscle pain in the rat in a naloxone-dependent manner. Arch Phys Med Rehabil. 2005;86:1736–40.

Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain. 1988;33:87–107.

Bernateck M, Becker M, Schwake C, Hoy L, Passie T, Parlesak A, Fischer MJ, Fink M, Karst M. Adjuvant auricular electroacupuncture and autogenic training in rheumatoid arthritis: a randomized controlled trial. Auricular acupuncture and autogenic training in rheumatoid arthritis. Forsch Komplementmed. 2008;15:187–93.

Cardenas DD, Jensen MP. Treatments for chronic pain in persons with spinal cord injury: a survey study. J Spinal Cord Med. 2006;29:109–17.

Chang FC, Tsai HY, Yu MC, Yi PL, Lin JG. The central serotonergic system mediates the analgesic effect of electroacupuncture on ZUSANLI (ST36) acupoints. J Biomed Sci. 2004;11:179–85.

Chen WN, Chen CC. Acid mediates a prolonged antinociception via substance P signaling in acid-induced chronic widespread pain. Mol Pain. 2014;10:30.

Chen XH, Han JS. Analgesia induced by electroacupuncture of different frequencies is mediated by different types of opioid receptors: another cross-tolerance study. Behav Brain Res. 1992;47:143–9.

Chen WK, Liu IY, Chang YT, Chen YC, Chen CC, Yen CT, Shin HS, Chen CC. Ca(v)3.2 T-type Ca^{2+} channel-dependent activation of ERK in paraventricular thalamus modulates acid-induced chronic muscle pain. J Neurosci. 2010;30:10360–8.

Chen WH, Hsieh CL, Huang CP, Lin TJ, Tzen JT, Ho TY, Lin YW. Acid-sensing ion channel 3 mediates peripheral anti-hyperalgesia effects of acupuncture in mice inflammatory pain. J Biomed Sci. 2011;18:82.

Chen WH, Tzen JT, Hsieh CL, Chen YH, Lin TJ, Chen SY, Lin YW. Attenuation of TRPV1 and TRPV4 expression and function in mouse inflammatory pain models using electroacupuncture. Evid Based Complement Alternat Med. 2012;2012:636848.

Chen WN, Lee CH, Lin SH, Wong CW, Sun WH, Wood JN, Chen CC. Roles of ASIC3, TRPV1, and NaV1.8 in the transition from acute to chronic pain in a mouse model of fibromyalgia. Mol Pain. 2014;10:40.

Cheng SJ, Chen CC, Yang HW, Chang YT, Bai SW, Chen CC, Yen CT, Min MY. Role of extracellular signal-regulated kinase in synaptic transmission and plasticity of a nociceptive input on capsular central amygdaloid neurons in normal and acid-induced muscle pain mice. J Neurosci. 2011;31:2258–70.

Clauw DJ. Fibromyalgia: a clinical review. JAMA. 2014;311:1547–55.

Dadabhoy D, Crofford LJ, Spaeth M, Russell IJ, Clauw DJ. Biology and therapy of fibromyalgia. Evidence-based biomarkers for fibromyalgia syndrome. Arthritis Res Ther. 2008;10:211.

DeSantana JM, Sluka KA. Central mechanisms in the maintenance of chronic widespread noninflammatory muscle pain. Curr Pain Headache Rep. 2008;12:338–43.

DeSantana JM, da Cruz KM, Sluka KA. Animal models of fibromyalgia. Arthritis Res Ther. 2013;15:222.

Di Stefano G, La Cesa S, Truini A, Cruccu G. Natural history and outcome of 200 outpatients with classical trigeminal neuralgia treated with carbamazepine or oxcarbazepine in a tertiary centre for neuropathic pain. J Headache Pain. 2014;15:34.

English B. Neural and psychosocial mechanisms of pain sensitivity in fibromyalgia. Pain Manag Nurs. 2014;15:530–8.

Galarza-Delgado DA, Azpiri-Lopez JR, Colunga-Pedraza IJ, Cardenas-de la Garza JA, Vera-Pineda R, Serna-Pena G, Arvizu-Rivera RI, Martinez-Moreno A, Wah-Suarez M, Garza Elizondo MA. Assessment of six cardiovascular risk calculators in Mexican mestizo patients with rheumatoid arthritis according to the EULAR 2015/2016 recommendations for cardiovascular risk management. Clin Rheumatol. 2017;36(6):1387–93.

Gan TJ, Habib AS. Adenosine as a non-opioid analgesic in the perioperative setting. Anesth Analg. 2007;105:487–94.
Gandhi R, Ryals JM, Wright DE. Neurotrophin-3 reverses chronic mechanical hyperalgesia induced by intramuscular acid injection. J Neurosci. 2004;24:9405–13.
Gerwin RD. Fibromyalgia tender points at examination sites specified by the American College of Rheumatology criteria are almost universally myofascial trigger points. Curr Pain Headache Rep. 2011;15:1–3.
Gim GT, Lee JH, Park E, Sung YH, Kim CJ, Hwang WW, Chu JP, Min BI. Electroacupuncture attenuates mechanical and warm allodynia through suppression of spinal glial activation in a rat model of neuropathic pain. Brain Res Bull. 2011;86:403–11.
Goldman N, Chen M, Fujita T, Xu Q, Peng W, Liu W, Jensen TK, Pei Y, Wang F, Han X, et al. Adenosine A1 receptors mediate local anti-nociceptive effects of acupuncture. Nat Neurosci. 2010;13:883–8.
Hamza MA, White PF, Ahmed HE, Ghoname EA. Effect of the frequency of transcutaneous electrical nerve stimulation on the postoperative opioid analgesic requirement and recovery profile. Anesthesiology. 1999;91:1232–8.
Han JS. Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. Trends Neurosci. 2003;26:17–22.
Häuser W, Walitt B, Fitzcharles MA, Sommer C. Review of pharmacological therapies in fibromyalgia syndrome. Arthritis Res Ther. 2014;16:201.
Hoeger-Bement MK, Sluka KA. Phosphorylation of CREB and mechanical hyperalgesia is reversed by blockade of the cAMP pathway in a time-dependent manner after repeated intramuscular acid injections. J Neurosci. 2003;23:5437–45.
Honjo H, Naya Y, Ukimura O, Kojima M, Miki T. Acupuncture on clinical symptoms and urodynamic measurements in spinal-cord-injured patients with detrusor hyperreflexia. Urol Int. 2000;65:190–5.
Hsu HC, Tang NY, Lin YW, Li TC, Liu HJ, Hsieh CL. Effect of electroacupuncture on rats with chronic constriction injury-induced neuropathic pain. Sci World J. 2014;2014:129875.
Huang CP, Chen HN, Su HL, Hsieh CL, Chen WH, Lai ZR, Lin YW. Electroacupuncture reduces carrageenan- and CFA-induced inflammatory pain accompanied by changing the expression of Nav1.7 and Nav1.8, rather than Nav1.9, in mice dorsal root ganglia. Evid Based Complement Alternat Med. 2013;2013:312184.
Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain. 1987;30:103–14.
Hurt JK, Zylka MJ. PAPupuncture has localized and long-lasting antinociceptive effects in mouse models of acute and chronic pain. Mol Pain. 2012;8:28.
Ikeuchi M, Kolker SJ, Sluka KA. Acid-sensing ion channel 3 expression in mouse knee joint afferents and effects of carrageenan-induced arthritis. J Pain. 2009;10:336–42.
Jang JY, Kim HN, Koo ST, Shin HK, Choe ES, Choi BT. Synergistic antinociceptive effects of N-methyl-D-aspartate receptor antagonist and electroacupuncture in the complete Freund's adjuvant-induced pain model. Int J Mol Med. 2011;28:669–75.
Johnson E. CDA assists in oral surgery performed under acupuncture anesthesia. Dent Assist. 1973;42:16.
Julius D, Basbaum AI. Molecular mechanisms of nociception. Nature. 2001;413:203–10.
Jung TG, Lee JH, Lee IS, Choi BT. Involvement of intracellular calcium on the phosphorylation of spinal N-methyl-D-aspartate receptor following electroacupuncture stimulation in rats. Acta Histochem. 2010;112:127–32.
Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, Lacroix-Fralish ML, et al. Coding of facial expressions of pain in the laboratory mouse. Nat Methods. 2010;7:447–9.
Lauria G, Faber CG, Merkies IS, Waxman SG. Diagnosis of neuropathic pain: challenges and possibilities. Expert Opin Med Diagn. 2012;6:89–93.
Li Q, Yue N, Liu SB, Wang ZF, Mi WL, Jiang JW, Wu GC, Yu J, Wang YQ. Effects of chronic electroacupuncture on depression- and anxiety-like behaviors in rats with chronic neuropathic pain. Evid Based Complement Alternat Med. 2014;2014:158987.

Lin JG, Lo MW, Wen YR, Hsieh CL, Tsai SK, Sun WZ. The effect of high and low frequency electroacupuncture in pain after lower abdominal surgery. Pain. 2002;99:509–14.
Liu Z, Wang W, Wu J, Zhou K, Liu B. Electroacupuncture improves bladder and bowel function in patients with traumatic spinal cord injury: results from a prospective observational study. Evid Based Complement Alternat Med. 2013;2013:543174.
Magrinelli F, Zanette G, Tamburin S. Neuropathic pain: diagnosis and treatment. Pract Neurol. 2013;13:292–307.
Mao JJ, Farrar JT, Bruner D, Zee J, Bowman M, Seluzicki C, DeMichele A, Xie SX. Electroacupuncture for fatigue, sleep, and psychological distress in breast cancer patients with aromatase inhibitor-related arthralgia: a randomized trial. Cancer. 2014;120:3744–51.
Martins DF, Bobinski F, Mazzardo-Martins L, Cidral-Filho FJ, Nascimento FP, Gadotti VM, Santos AR. Ankle joint mobilization decreases hypersensitivity by activation of peripheral opioid receptors in a mouse model of postoperative pain. Pain Med. 2012;13:1049–58.
Mogil JS. Animal models of pain: progress and challenges. Nat Rev Neurosci. 2009;10:283–94.
Moon HJ, Lim BS, Lee DI, Ye MS, Lee G, Min BI, Bae H, Na HS, Kim SK. Effects of electroacupuncture on oxaliplatin-induced neuropathic cold hypersensitivity in rats. J Physiol Sci. 2014;64:151–6.
Nam JL, Takase-Minegishi K, Ramiro S, Chatzidionysiou K, Smolen JS, van der Heijde D, Bijlsma JW, Burmester GR, Dougados M, Scholte-Voshaar M, et al. Efficacy of biological disease-modifying antirheumatic drugs: a systematic literature review informing the 2016 update of the EULAR recommendations for the management of rheumatoid arthritis. Ann Rheum Dis. 2017;76:1113.
Nayak S, Shiflett SC, Schoenberger NE, Agostinelli S, Kirshblum S, Averill A, Cotter AC. Is acupuncture effective in treating chronic pain after spinal cord injury? Arch Phys Med Rehabil. 2001;82:1578–86.
Nielsen AN, Mathiesen C, Blackburn-Munro G. Pharmacological characterisation of acid-induced muscle allodynia in rats. Eur J Pharmacol. 2004;487:93–103.
Parker AJ, Wessely S, Cleare AJ. The neuroendocrinology of chronic fatigue syndrome and fibromyalgia. Psychol Med. 2001;31:1331–45.
Politis MJ, Korchinski MA. Beneficial effects of acupuncture treatment following experimental spinal cord injury: a behavioral, morphological, and biochemical study. Acupunct Electrother Res. 1990;15:37–49.
Pratt D, Fuchs PN, Sluka KA. Assessment of avoidance behaviors in mouse models of muscle pain. Neuroscience. 2013;248:54–60.
Price MP, McIlwrath SL, Xie J, Cheng C, Qiao J, Tarr DE, Sluka KA, Brennan TJ, Lewin GR, Welsh MJ. The DRASIC cation channel contributes to the detection of cutaneous touch and acid stimuli in mice. Neuron. 2001;32:1071–83.
Quintero IB, Araujo CL, Pulkka AE, Wirkkala RS, Herrala AM, Eskelinen EL, Jokitalo E, Hellstrom PA, Tuominen HJ, Hirvikoski PP, Vihko PT. Prostatic acid phosphatase is not a prostate specific target. Cancer Res. 2007;67:6549–54.
Riva R, Mork PJ, Westgaard RH, Okkenhaug Johansen T, Lundberg U. Catecholamines and heart rate in female fibromyalgia patients. J Psychosom Res. 2012;72:51–7.
Ross GR, Gade AR, Dewey WL, Akbarali HI. Opioid-induced hypernociception is associated with hyperexcitability and altered tetrodotoxin-resistant Na+ channel function of dorsal root ganglia. Am J Physiol Cell Physiol. 2012;302:C1152–61.
Roth T, Arnold LM, Garcia-Borreguero D, Resnick M, Clair AG. A review of the effects of pregabalin on sleep disturbance across multiple clinical conditions. Sleep Med Rev. 2014;18:261–71.
Ryu JW, Lee JH, Choi YH, Lee YT, Choi BT. Effects of protein phosphatase inhibitors on the phosphorylation of spinal cord N-methyl-D-aspartate receptors following electroacupuncture stimulation in rats. Brain Res Bull. 2008;75:687–91.
Sharma NK, Ryals JM, Gajewski BJ, Wright DE. Aerobic exercise alters analgesia and neurotrophin-3 synthesis in an animal model of chronic widespread pain. Phys Ther. 2010;90:714–25.
Shields SD, Eckert WA III, Basbaum AI. Spared nerve injury model of neuropathic pain in the mouse: a behavioral and anatomic analysis. J Pain. 2003;4:465–70.

Skyba DA, King EW, Sluka KA. Effects of NMDA and non-NMDA ionotropic glutamate receptor antagonists on the development and maintenance of hyperalgesia induced by repeated intramuscular injection of acidic saline. Pain. 2002;98:69–78.
Sluka KA, Kalra A, Moore SA. Unilateral intramuscular injections of acidic saline produce a bilateral, long-lasting hyperalgesia. Muscle Nerve. 2001;24:37–46.
Sluka KA, Rohlwing JJ, Bussey RA, Eikenberry SA, Wilken JM. Chronic muscle pain induced by repeated acid injection is reversed by spinally administered mu- and delta-, but not kappa-, opioid receptor agonists. J Pharmacol Exp Ther. 2002;302:1146–50.
Sluka KA, Price MP, Breese NM, Stucky CL, Wemmie JA, Welsh MJ. Chronic hyperalgesia induced by repeated acid injections in muscle is abolished by the loss of ASIC3, but not ASIC1. Pain. 2003;106:229–39.
Sluka KA, Radhakrishnan R, Benson CJ, Eshcol JO, Price MP, Babinski K, Audette KM, Yeomans DC, Wilson SP. ASIC3 in muscle mediates mechanical, but not heat, hyperalgesia associated with muscle inflammation. Pain. 2007;129:102–12.
Smith MT, Moore BJ. Pregabalin for the treatment of fibromyalgia. Expert Opin Pharmacother. 2012;13:1527–33.
Sniezek DP, Siddiqui IJ. Acupuncture for treating anxiety and depression in women: a clinical systematic review. Med Acupunct. 2013;25:164–72.
Sowa NA, Street SE, Vihko P, Zylka MJ. Prostatic acid phosphatase reduces thermal sensitivity and chronic pain sensitization by depleting phosphatidylinositol 4,5-bisphosphate. J Neurosci. 2010;30:10282–93.
Steen KH, Steen AE, Kreysel HW, Reeh PW. Inflammatory mediators potentiate pain induced by experimental tissue acidosis. Pain. 1996;66:163–70.
Stein C. Opioid receptors on peripheral sensory neurons. Adv Exp Med Biol. 2003;521:69–76.
Trugman JM, Palmer RH, Ma Y. Milnacipran effects on 24-hour ambulatory blood pressure and heart rate in fibromyalgia patients: a randomized, placebo-controlled, dose-escalation study. Curr Med Res Opin. 2014;30:589–97.
Tu WZ, Cheng RD, Cheng B, Lu J, Cao F, Lin HY, Jiang YX, Wang JZ, Chen H, Jiang SH. Analgesic effect of electroacupuncture on chronic neuropathic pain mediated by P2X3 receptors in rat dorsal root ganglion neurons. Neurochem Int. 2012;60:379–86.
Valim V, Natour J, Xiao Y, Pereira AF, Lopes BB, Pollak DF, Zandonade E, Russell IJ. Effects of physical exercise on serum levels of serotonin and its metabolite in fibromyalgia: a randomized pilot study. Rev Bras Reumatol. 2013;53:538–41.
van Hecke O, Austin SK, Khan RA, Smith BH, Torrance N. Neuropathic pain in the general population: a systematic review of epidemiological studies. Pain. 2014;155:654–62.
Vierck CJ Jr. Mechanisms underlying development of spatially distributed chronic pain (fibromyalgia). Pain. 2006;124:242–63.
Walder RY, Rasmussen LA, Rainier JD, Light AR, Wemmie JA, Sluka KA. ASIC1 and ASIC3 play different roles in the development of hyperalgesia after inflammatory muscle injury. J Pain. 2010;11:210–8.
Wang B, Tang J, White PF, Naruse R, Sloninsky A, Kariger R, Gold J, Wender RH. Effect of the intensity of transcutaneous acupoint electrical stimulation on the postoperative analgesic requirement. Anesth Analg. 1997;85:406–13.
Wang L, Zhang Y, Dai J, Yang J, Gang S. Electroacupuncture (EA) modulates the expression of NMDA receptors in primary sensory neurons in relation to hyperalgesia in rats. Brain Res. 2006;1120:46–53.
Widerstrom-Noga EG, Felipe-Cuervo E, Yezierski RP. Chronic pain after spinal injury: interference with sleep and daily activities. Arch Phys Med Rehabil. 2001;82:1571–7.
Wong AM, Leong CP, Su TY, Yu SW, Tsai WC, Chen CP. Clinical trial of acupuncture for patients with spinal cord injuries. Am J Phys Med Rehabil. 2003;82:21–7.
Wu Y, Liu C, Chen Q. Effect of acupuncture on enzymology of motor neuron of anterior horn of experimental spinal cord injury in rats. Zhongguo Zhong Xi Yi Jie He Za Zhi. 1999;19:740–2.

Xing GG, Liu FY, Qu XX, Han JS, Wan Y. Long-term synaptic plasticity in the spinal dorsal horn and its modulation by electroacupuncture in rats with neuropathic pain. Exp Neurol. 2007;208:323–32.

Yokoyama T, Maeda Y, Audette KM, Sluka KA. Pregabalin reduces muscle and cutaneous hyperalgesia in two models of chronic muscle pain in rats. J Pain. 2007;8:422–9.

Zhang RX, Wang L, Wang X, Ren K, Berman BM, Lao L. Electroacupuncture combined with MK-801 prolongs anti-hyperalgesia in rats with peripheral inflammation. Pharmacol Biochem Behav. 2005;81:146–51.

Zhu Z. Effects of electroacupuncture on laminin expression after spinal cord injury in rats. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2002;22:525–7.