Effects of Electroacupuncture on Cutaneous Wound Healing in Rats.

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ABSTRACT

The effects of electroacupuncture (EA) on the healing of full thickness skin incision wounds were examined in the rat. Epidermal wound healing was measured using a non-invasive quantitative method based on the increasing electrical resistance of healing skin. Dermal healing was assessed by measuring the wound breaking strength. We report that EA accelerates epidermal wound healing by 55% and dermal healing by 22% when compared to controls. In addition, EA effects on epidermal wound healing are abolished by nerve block with subcutaneous lidocaine injection. Thus this implicates peripheral nerves in the EA effects. Due to technical reasons the lidocaine blockade could not be tested on dermal effects of EA. This is the first study demonstrating that EA potentiates epidermal and dermal wound healing and that some of this effect is mediated by nerves.

INTRODUCTION

The study of wound healing is often an overlooked area of medical research. However, the implications of improved wound healing are far reaching in terms of both basic scientific research and cost of medical care. Indolent or chronic wounds are a serious problem associated with 3% (Petersen and Bittman 1971) of all hospital patients and presents healing problems in 30% of all spinal cord injury patients (Young and Burns 1982). In addition, poor wound healing in diabetics accounts for 50 to 80% of all lower extremity amputations (Most and Sinnock 1983, van Houtum *et al.* 1996, Lavery *et al.* 1996). Thus, wound care in chronic and acute wounds is of paramount clinical importance.

Alternative therapies for human disease have become increasingly popular in the past few decades. A recent study published in the New England Journal of Medicine (Eisenberg *et al.* 1993) showed that in 1990, Americans made more visits to unconventional therapists than they did to conventional therapists. Acupuncture was among the most popular unconventional therapy. In a recent study, it was reported that about 64% of US medical schools offer elective courses in alternative medicine (Wetzel *et al.* 1998). Research into the mechanisms of unconventional therapies must be scientifically examined before they can be accepted in current conventional medical practice.

In China, acupuncture has been used to treat skin wounds for over 1000 years, but the physiological mechanisms have not been studied (Stux and Pomeranz 1995). This thesis examines the effects of electroacupuncture (EA) on the healing of incisional wounds in the rat.

LITERATURE REVIEW

Physiology of Acupuncture

Acupuncture has been used in China for over 2000 years to treat various diseases. It was introduced into Europe in the seventeenth century and has become increasingly popular in the west. Although the therapeutic effects of acupuncture have been known for some time, the lack of a physiological basis for it has been one of the main reasons why it has not been more accepted in western practice.

In the past few decades, scientific research has shed some light on the physiological basis of acupuncture. The physiology of acupuncture on pain, hypertension, stroke rehabilitation, epilepsy, and addictions has been studied (for review see Anderson 1993, Wu 1990, Andersson and Lundeberg 1995, Yao 1993).

Pain

Acupuncture analgesia has been the most extensively studied aspect of acupuncture. The neurophysiology of acupuncture has been well documented (for a review see Stux and Pomeranz 1995). One of the big breakthroughs in acupuncture research occured when it was found that EA given for 20 minutes increased pain thresholds in mice, and that this effect was blocked with naloxone, an opioid antagonist (Pomeranz and Chiu 1976). This result implicated endogenous pain relieving

mechanisms mediated by the opioid system. This observation was confirmed in a human study that found increased levels of endorphins in cerebral spinal fluid after acupuncture (Sjölund *et al.* 1977).

Acupuncture analgesia is mediated by the central nervous system (CNS) at 3 levels. They are: the spinal cord, brain stem, and pituitary. Chan and Fung (1975) performed one of the first experiments suggesting that the spinal cord is involved in mediating acupuncture analgesia. The authors concluded that the acupuncture effects were mediated by a polysynaptic pathway. Pomeranz and Cheng (1979) found that noxious stimuli in the cat evoked single unit responses in the dorsal horn, and EA reduced these responses. In addition, naloxone blocked the EA suppression. Han and Xie (1984) found that intrathecal injection of anti-dynorphin antibody reduced acupuncture analgesia. Similarly, Bing *et al.* (1991) recently demonstrated that acupuncture activates enkephalinergic neurons in the spinal cord that could mediate presynaptic inhibition of incoming pain signals. In experiments using antibodies against enkephalin or β-endorphin, the authors found that enkephalin plays an important role EA analgesia in the spinal cord whereas β-endorphin does not (Han *et al.* 1984b). These findings implicate opioid mechanisms for acupuncture analgesia in the spinal cord.

Deep brain stimulation of the midbrain has been used extensively for the treatment of chronic pain in human patients (for review see Kumar *et al.* 1997). Endorphinergic neurons in the periaqueductal grey (PAG; part of the mid brain) send excitatory input to the serotonergic raphe nucleus of the medulla. This monoaminergic

system can then cause inhibition of pain in the spinal cord via the dorsolateral funniculus (for review see Basbaum and Fields 1984). The endogenous monoamine analgesia system in the midbrain plays in important role in mediating acupuncture analgesia. Acupuncture stimulation was found to evoke potentials in PAG (Takeshige *et al.* 1992). Depletion of monoamines inhibited EA (Cheng and Pomeranz 1981). Recent experiments suggest that cholecystokinin (CCK) acts to inhibit EA analgesic effects (Han *et al.* 1986), and that CCK was found to be higher in the PAG of rats who show poor EA analgesia (Tang *et al.* 1997). In the same study, preventing CCK expression by injecting an antisense CCK vector, caused poor responding EA rats to be converted to good responding EA rats. These results suggest that the monoamergic system from the PAG and midbrain is involved in EA analgesia.

Clinically, acupuncture analgesia takes several minutes to take place. This suggests a hormonal aspect to acupuncture. Hypophysectomy in mice was found to abolish EA analgesia (Pomeranz *et al.* 1977). In addition, c-*fos* expression was found to be increased in pituitary anterior lobe and arcuate nucleus of the hypothalamus (Pan *et al.* 1994). More recently, c-*fos* expression after EA was found to be localized in pituitary cells immunoreactive for adrenocorticotropic hormone or β-endorphin and not any other pituitary cells (Pan *et al.* 1996).

Cardiovascular Disorders

The effects of acupuncture on the cardiovascular system and that of exercise are very similar. Both are known to release opioids (Fontana *et al.* 1994; Sjölund *et al.* 1977) and both have 2 distinct phases: excitation and depression. During exercise, the sympathetic nervous system is stimulated leading to an increased heart rate, cardiac output, peripheral vasoconstriction, and increases in blood pressure. After exercise, sympathetic inhibition as seen by a reduction in blood pressure and postganglionic muscle sympathetic activity occurs (Floras *et al.* 1989). Similarly, EA in humans shows this initial excitation and post stimulatory inhibition of the sympathetic nervous system (Dyerhag *et al.* 1997). These biphasic EA and exercise effects on the autonomic system appear to be mediated reflexively centrally via opioids (Reid *et al.* 1984). Clinically, acupuncture has been shown to decrease blood pressure in hypertensive patients when compared to sham stimulated hypertensive patients (Williams *et al.* 1991). Nishijo *et al.* (1997) showed that acupuncture stimulation decreases heart rate in healthy humans. The mechanism they proposed was an increase in cardiac vagal activity and a decrease in cardiac sympathetic activity. In addition, Chiu *et al.* (1997) demonstrated that acupuncture given for 30 minutes resulted in a decrease in arterial blood pressure and heart rate. The authors suggest that anti-hypertensive effects were attributed to a decrease in plasma renin.

The effects of EA on blood pressure are mediated by the central nervous system (Lovick *et al.* 1995; Wang *et al.* 1988). Stimulation of soma

reduction in the activity of neurons in the dorsal vagal nucleus and nucleus ambiguus. The reduction of these cardiomotor neurons accounted for the reduction in blood pressure (Wang *et al.* 1988). In the midbrain, stimulation of the PAG causes a pressor response (i.e. tachycardia and vasodilation). Lovick *et al.* (1995) showed that stimulation of the peroneal nerve at intensities sufficient to recruit Aβ and Aδ fibres caused the pressor response to be attenuated by 50%. These results suggest that EA stimulation causes a central sympathetic inhibition which can result in decreases in blood pressure.

Richter *et al.* (1991) demonstrated that acupuncture had beneficial effects in patients with angina pectoris. In that study, acupuncture or placebo was given for 4 weeks. During that time period, the anginal attacks were reduced compared to placebo, there was an increase in work performance and decreases in pain at high levels of bicycling compared to placebos, and additionally, the ST-segment intervals were depressed at maximal workloads compared to placebos. Life quality questionnaire forms confirmed the well being of the acupuncture patients. Similar effects on angina pectoris were seen with TENS (Mannheimer *et al.* 1985). In contrast with acupuncture analgesia, naloxone had no effect (Mannheimer *et al.* 1989).

Stroke Rehabilitation

Hu *et al.* (1993) showed that acupuncture resulted in an improved recovery on day 28 and 90 in acute partial ischemic stroke patients compared to standard rehabilitation procedure patients. This result was confirmed by Magnusson *et al.* (1994)

who found that in severe hemiparetic stroke patients, acupuncture with physical and occupational therapy performed better than those who received only physical and occupational therapy.

Neurological Disorders

As discussed earlier, EA affects the monoamergic system in the brain stem, and as such would imply possible effects of acupuncture on central neurological disorders such as depression. Catecholamines were implicated in affective disorders such as depression and mania in the classical paper by Schildkraut (1965). Han (1986) compared the effects of EA and amitriptyline (blocks re-uptake of serotonin) on depression patients. After 5 weeks of treatment, there were significant decreases in depression (as assessed by the Hamilton Depression Rating Scale) in the EA group and amitriptyline group (-55% vs. -52%). There was no difference between the 2 experimental groups. This study showed that EA is at least equally effective as the traditional treatment. However, the amitriptyline group showed the usual side effects of dizziness, fatigue, palpitation, and dry mouth whereas the EA treatment showed no side effects.

Acupuncture has also been reported to suppress penicillin induced epileptiform discharges in the sensory-motor cortex (Wu 1990). In addition, acupuncture was found to be beneficial in the treatment of recidivist alcoholism (Bullock *et al.* 1989).

The above is only a brief summary of the acupuncture literature. It is presented here to reflect the extent and magnitude of possible acupuncture effects. Acupuncture for wound healing, the subject of this thesis, will be discussed below.

Cutaneous Wound Healing

Wound healing is a complex process which involves the co-ordination of various cell types and their cytokines or mediators. Wound healing is generally divided into 3 overlapping stages, which are: inflammation, tissue formation (proliferative phase), and tissue remodeling (Clarke 1996).

Inflammation

The inflammatory phase of wound healing begins once the injury is created and persists for 10 days thereafter in humans (Clarke 1996). Two general cell types are involved in the inflammatory phase, platelets and leukocytes.

Platelets

Tissue injury leads to the disruption of blood vessels and the extravasation of red blood cells and other constituents of the blood. The most important cell in the early phase of inflammation is the platelet (Kirsner and Eaglestein 1993). When the subepithelial collagen is exposed, platelets adhere, become activated and release their α and dense granules, thus forming a hemostatic plug (Santoro 1986). The contents of the platelet granules include adenosine diphosphate (ADP), thromboxane, A2, serotonin, fibrinogen, fibronectin, thrombospondin, and Von Willebrande factor VIII (Plow *et al.* 1985). Fibrinogen, fibronectin and thrombospondin act as ligands for platelet aggregation, while von Willebrand factor mediates platelet adhesion to collagen (Plow *et al.* 1986).

The formation of the fibrin clot results in the provisional matrix, which allows a medium for the migration of monocytes, fibroblasts, and kerintocytes into the wound environment (Yamada and Clarke 1996).

Leukocytes

Neutrophils and monocytes infiltrate the wound at the same time. Since neutrophils are greater in number, they are the predominant leukocyte in this early phase of inflammation. Both neutrophils and monocytes are attracted to the wound site by various chemo attractants. Some of these chemotactic factors include: kallikrein, fibrinopeptides (released from fibrinogen when cleaved by thrombin), fibrin degradation products (produced by plasmin degradation of fibrin), leukotriene B4 (released by activated neutrophils), platelet activating factor (released by endothelial cells and activated neutrophils), formyl methionyl peptides cleaved from bacterial proteins, and platelet derived growth factor (PDGF). Neutrophils at the wound site destroy contaminating bacteria via phagocytosis and subsequent enzymatic degradation (for review see Riches 1996).

Monocytes eventually become the dominant leukocyte in the wound after two days. Once in the wound they are transformed to tissue macrophages. Tissue macrophages, like the neutrophils also decontaminate the wound. They digest, phagocytize, and kill pathogenic organisms, scavenge tissue debris, and destroy any remaining neutrophils (Riches 1996).

After the phagocytes decontaminate the wound, angiogenisis and tissue granulation are allowed to proceed. In essence, the tissue macrophages play a pivotal role in the transition between inflammation and repair. Aside from direct decontamination of the wound, macrophages synthesize and release a host of chemical mediators. Some of these include: PDGF, fibroblast growth factor (FGF), transforming growth factor (TFG) α and β (Falanga *et al.* 1988). These cytokines are important in inducing cell migration and proliferation and extracellular matrix production, important for epidermal and dermal repair.

Tissue Formation

The second phase of wound healing involves reepithelialization, tissue granulation, fibroplasia, angiogenesis, and wound contraction.

In humans, the epidermis responds to the injury within 24 hours of the injury (Woodley 1996). Epithelial cells migrate from residual epithelial structures across the wound. The classical leap frog model of reepithelialization is characterized by epidermal cells migrating two or three cell lengths while sliding or rolling over previously implanted epidermal cells. These migrating cells in turn become implanted and other successive cells migrate over these. The resulting epidermal layer forms four to six cells thick. (Krawczyk 1971). Cell migration rather than cell division is crucial in the early stages of healing wounds (Krawczyk 1977). In experimental studies, blocking cell division had no effect on epithelial cell movement or wound closure (Dunlap and Donaldson 1978). Along with cell migration, the free edge epithelial cells undergo a

phenotypic alteration. The epithelial cells retract intercellular tonofilaments, the desmosomes (structures that interlink epithelial cells) dissolve, and there is the formation of peripheral cytoplasmic actin filaments (Gabbiani *et al.* 1978). As a result of these phenotypic changes, the wound epithelial cells have lateral motility.

In humans, one to two days after the injury, epithelial cells begin to proliferate (Krawczyk 1971). The stimulus for the epithelial cells to proliferate has not been fully worked out. A variety of growth factors have been implicated (refer to table 1). The epidermal growth factor (EGF) family is thought to play an important role in stimulating epithelial cell proliferation. The EGF receptors bind to transforming growth factor α (TGF-α), heparin binding epidermal growth factor (HB-EGF). In addition, fibroblast growth factor (FGF) 1 and 2 are thought to mediate epithelial cell proliferation. (Tarnuzzer *et al.* 1997).

In humans, granulation tissue begins to form approximately 4 days post injury (Clarke 1996). The granular appearance of the tissue is due to new capillary formation. Fibroblasts, migrate into the wound area and under the influence of cytokines and mitogenic factors form the fibroplasia. The fibroplasia consists of fibroblasts and loose extracellular matrix. *In vivo* studies suggest that growth factors are active in fibroplasia formation (Quaglino 1990, Matsuoka and Grotendorst 1989, Montesano and Orci 1989). Fibronectin forms part of the extracellular matrix, then fibroblasts use protein strands for movement. Once the fibroblasts migrate into the wound area, they switch to a prote

Table 1 Growth factors in wound healing. (Modified from Clarke 1996)

Growth Factor	Source	Effect
Fibroblast growth factor-1 and -2 (FGF)	Macrophages	Fibroblast and epidermal cell proliferation; angiogenesis
Insulinlike growth factor (IGF)	Macrophages	Progression factor for cell proliferation
Keratinocyte growth factor (KGF), also known as FGF-7	Macrophages	Keratinocyte proliferation
Platelet derived growth factor (PDGF) including isoforms AA, AB, and BB	Platelets	Fibroblast chemotaxis, proliferation and contraction
Transforming growth factor α (TGF-α) and epidermal growth factor (EGF)	Macrophages, monocytes, lymphocytes, and keratinocytes	Reepithelialization
Transforming growth factor β (TGF-β) icluding isoforms β1, β2, and β3	Platelets	Fibroblast chemotaxis; extracellular matrix deposition; protease inhibitor secretion
Vascular endothelial growth factor (VGEF)	Macrophages, epidermis	Vascular permeability; angiogenesis

synthesis mode in which they produce large quantities of collagen. Once an abundant collagen matrix is formed in the wound area, fibroblast activity decreases (Clarke 1996).

Afterwards, the fibroblasts undergo a phenotypic change and become actin rich myofibroblasts. These cells orient themselves in the line of wound contraction. *In vitro* stimulation by a number of mediators causes muscle-like contractions. Some of these

mediators include angiotensin, epinephrine (E), norepinephrine (NE), 5-hydroxytryptamine, prostaglandins (PG), and bradykinin (BK) (Kirsner and Eaglestein 1993).

Angiogenisis refers to the growth of new blood vessels. This is important for the basic nutrition of the healing wound. New capillary formation arises from blood vessels adjacent to the wound. FGF stimulates endothelial cells which line the blood vessels to release plasminogen activator (PA) and pro collagenase. PA liberates plasmin from the plasma derived plasminogen and activates collagenase. Both of these enzymes degrade the basement membrane of the blood vessel. Heparin and fibronectin fragments stimulate the endothelial cells to project pseudopodia through the basement membrane and in turn form new blood vessels (for reviews see Clarke 1996, Madri *et al.* 1996).

Tissue Remodeling

The third phase of wound healing begins at 20 days in humans and involves depositing matrix materials and their subsequent change over time. Fibronectin, hyluronic acid, proteoglycans, and collagen are deposited during the repair and serve as a scaffold for cellular migration and re-enforcement of the healed wound. In humans, one month post injury, the dermis resembles the preinjury state, and the wound repair is considered complete. The newly formed wound however, does not reach its preinjury tensile strength (Kirsner and Eaglestein 1993).

Nerves and Their Potential Role in Wound Healing

The influence of nerves on limb regeneration has been well documented. Liversage and McLaughlin (1984) showed that denervated adult newts did not regenerate their limbs, whereas normal adult newts regenerated their limbs. Since wound healing is tissue regeneration it seems logical that there would be a neural influence on healing. Surprisingly, not much work has been done in this area. One study that examined this aspect found that wound contraction was impaired in denervated rats (Engin *et al.* 1998). Recent evidence from our laboratory found that surgical denervation and chemical sympathectomy inhibit wound healing (submitted).

Models Which Implicate Nerves and Wound Healing

The role of nerves in wound healing has only been recognized in the past few years. Traditionally, wound healing experiments have been carried out in *in vitro* experiments where some of the endogenous systems have not been intact. Thus, the role of nerves may have been neglected for this reason.

One of the trademark features of diabetes is peripheral neuropathy. Human diabetic patients have characteristic decreases in nerve conduction velocity (Ohgaki *et al.* 1998). Similarly in experimental diabetic rats, a reduction in nerve conduction velocity is seen (Cameron *et al.* 1986). Diabetic patients who have peripheral neuropathy exhibit poor wound healing. In fact, 50 to 80% of lower extremity amputations are due to poor wound healing in diabetics (Most and Sinnock 1983, van Houtum *et al.* 1996, Lavery *et*

al. 1996). Diabetic patients have a 5 to 46 fold greater risk for lower extremity amputation than non-diabetic patients do (Armstrong *et al.* 1997, Most and Sinnock 1983). In addition to neuropathy, diabetics have blood flow disturbances (for a review see Levin 1995). Given the observation that diabetics experience impaired wound healing, neuropathy, leading to poor blood flow can be seen as a factor contributing to the impairment. Neuropathy in diabetic patients is thought to arise from long term insulin deficiency and or hyperglycemia. This is related to inositol transport into the nerve cells. A reduced myoinositol uptake and metabolism and consequent reduction in Na^+-K^+ ATPase activity has been shown to be impaired in streptozotocin induced diabetic rats (Greene 1986). This reduction of Na^+-K^+ ATPase activity results in an increased intracellular Na^+ concentration, and axonal swelling which cause decreases in nerve conduction velocity. The observation that diabetics exhibit peripheral neuropathy and poor wound healing suggests a neural influence on wound healing.

The skin flap survival model is a common ischemic wound healing model used to examine the role of nerves. C-fibre sensory denervation of skin flaps in rats with capsaicin was shown to cause a 30% reduction in flap survival compared to controls (Kjartansson *et al.* 1986). More recently, incisional wounds have been studied. Engin *et al.* (1995) found that denervation caused a reduction in wound contraction in rats. Our laboratory has found that denervation causes a reduction in the rate of epidermal healing (submitted).

Taken together, these studies suggest that nerves may play a role in normal wound healing.

Neurogenic Inflammation

Neurogenic inflammation, as the name implies, is inflammation promoted by nerves. One of the first studies which showed that nerves can cause vasodilatation (an important aspect of neurogenic inflammation) was performed at the turn of the century (Bayliss 1901). Bayliss showed that central stimulation of nerves in the dorsal horn could cause vasodilatation in the periphery. The notion of an axon reflex was introduced by Bruce in 1913. He showed that edema due to mustard oil application disappears after sensory nerve transection.

Neurogenic inflammation in the scientific literature remained almost untouched until recently when the scientific community took a renewed interest. The first of these renewed experiments on neurogenic inflammation was conducted by Jansco *et al.* (1967). It was found that antidromic stimulation of primary afferents caused an increase in vascular permeability in rat skin. Gamillscheg *et al.* (1984) found that rats pretreated with capsaicin showed a permanent deficit of carrageenan induced edema. This showed that neurogenic inflammation was dependent on unmyelinated primary afferents. The eleven amino acid peptide substance P (SP) found in unmyelinated afferents has been the focus of much attention in neurogenic inflammation. SP by itself can elicit signs of inflammation, including vasodilatation, plasma extravasation (PE), and mast cell degranulation (Saria 1984). Calcitonin gene related peptide (CGRP), a potent vasodilator

(Brain *et al.* 1985) is known to be co-released with SP from sensory afferents (antidromically) and thus potentiating neurogenic inflammation (Holzer, 1992; Franco-Cereceda *et al.* 1987). It is well accepted that high doses of capsaicin depletes the SP and CGRP, thus impeding inflammation.

Sympathetic Nervous System

The effects of the sympathetic nervous system, and more precisely, the sympathetic postganglionic neurons (SPGNs) on neurogenic inflammation have been extensively studied in the rat knee joint model (for a review see Coderre *et al.* 1989). Briefly, the rat knee joint allows for an ideal study of the interactions between SPGNs and primary afferent nerves and their effects on vascular permeability. The synovial membrane has a rich blood supply and nerve source. By injecting a plasma protein dye such as Evan's blue into the blood supply to the joint, evidence for plasma extravasation (PE) can be seen when a sample of synovial fluid is withdrawn and Evan's blue concentration is measured. Pharmacological manipulation of the nerve source and blood vessels allows the investigator to examine the effects of various drugs.

Coderre *et al.* (1989) found that PE could be induced by primary afferent stimulation (low dose capsaicin), and SPGN stimulation (low dose 6-hydroxydopamine, 6-OHDA). Chronic pretreatment with 6-OHDA, which denervates the sympathetic nerves resulted in a diminished PE elicited by low dose capsaicin. The authors further showed that SPGN dependant production of prostaglandins contributes to the increase in vascular permeability produced by activation of primary afferents. Thus, the SPGN may

play a more crucial role than primary afferents in neurogenic inflammation. Further studies showed that bradykinin induces PE, and that prostaglandin E_2 (PGE_2), ATP, and adenosine A_2 receptor agonists enhanced BK induced PE (Greene *et al.* 1993).

Thus, two basic neural mediators of inflammation have been studied. These are the antidromic stimulation of the primary afferent and the SPGN. The SPGN can be stimulated in two ways: orthodromic stimulation directly on the SPGN, or reflexively via activation of somatic afferents to the spinal cord (i.e. somatosympathetic reflexes, see below).

Somatosympathetic Reflexes

Modifying sympathetic activity via somatic input to the spinal cord (i.e. somatosympathetic reflexes) might play a role in wound healing (as well as in EA effects). Somatosympathetic reflexes are important in wound healing since reflexive vasodilatation will be able to augment wound healing. The sympathetic nervous system plays a role in wound healing since experiments in our laboratory have found that sympathectomy impairs wound healing (submitted).

The concept that somatic input can modify the autonomic nervous system is not a new one. A century ago, Carl Ludwig and coworkers found that electrical stimulation of limb nerves induced changes in blood pressure (as cited by Sato and Schmidt 1973). A common method of examining these somatosympathetic reflexes involves stimulating a

peripheral nerve and recording changes in spontaneous activity in the white rami (sympathetic preganglionic neurons) or sympathetic post ganglionic neurons. Jänig *et al.* (1972) found that cutaneous postganglionic neurons exhibit tonic discharges and they are under the influence of peripheral nerves. Peripheral afferent nerve stimulation ellicits two responses, an early increase in spontaneous activity (latency of 350 msec) and a post stimulatory inhibition of spontaneous activity (latency of 1000 msec). Stimulating the afferent nerve at intensities sufficient to recruit group II (Aβ) and III (Aδ) afferents caused both responses, while stimulating at group IV (C-fibre) strength potentiated the inhibition of spontaneous activity. The early component remains intact after spinalization and thus seems to be a spinal reflex. The longer latency reflex disappears after spinalization and thus appears seems to be a supraspinal reflex (Kimura *et al.* 1996).

Somatosympathetic reflexes have also been examined in humans. Blumberg and Wallin (1987) found that peroneal nerve stimulation can induce reflexive vasodilatation in the ipsilateral and contralateral foot. The reflex vasodilation was only present at painful stimulus intensities, thus the authors concluded that afferent Aδ fibres can induce this sympathetic reflex.

EA and Transcutaneous Electrical Nerve Stimulation (TENS) in Ischemic Wound Healing

The effects of TENS and EA on ischemic skin flaps have been well studied. Table 2 provides a survey of various studies that examined EA or electrical nerve stimulation and ischemic wound healing. The proposed mechanism of the observed effects of EA and TENS in ischemic skin flaps and human ulcers is that of improved skin circulation through the release of SP and CGRP by antidromic stimulation of primary afferents. The increase in skin blood flow due to these peptides has been demonstrated in the rat (Jansen *et al.* 1989). In support of this, CGRP injection has been shown to increase skin flap survival in pigs (Heden *et al.* 1989). Kjartansson *et al.* (1987) reported that reserpine pretreatment (which depletes catecholamines from adrenergic neurons, including sympathetic ganglia) helped with skin flap survival. This finding emphasizes the fact that reduction in blood flow is the major obstacle in skin flap survival. This is not the case with the full thickness incisional wounds studied in this thesis. No work on the role of SPGNs in EA and TENS on full thickness incision wound healing has been done.

Table 2 Survey of electroacupuncture (EA) and transcutaneous electrical nerve stimulation (TENS) studies on wound healing.

Study	Paradigm	Results
Jansen *et al.* 1989b	EA in rat skin flaps	Control skin flap survival = 43.1% EA survival = 92%
Jansen *et al.* 1989a	EA rat skin flap	Blood flow border increased by 66%
Niina et al. 1997	EA and TENS in rats	EA: no effect Control for TENS skin flap survival = 45% TENS survival = 75%
Brown and Gogia 1986	TENS in rabbit skin flaps	no difference between experimental and control groups *
Kaada 1983	TENS in human ischemic ulcers	Promotion of healing
Kaada 1982	TENS in human peripheral ischemia	Vasodilatation for 4-8 hrs
Kjartansson *et al.* 1988a	TENS in rat skin flaps	Blood flow border increased by 100%
Kjartansson *et al.* 1988b	TENS in rat skin flaps	Control skin flap survival = 33-45% TENS survival = 95%
Kjartansson and Lundeberg 1990	TENS in human skin flaps	Placebo stimulation: no effect on blood flow increases TENS: increase in blood flow
Kloth and Feedar 1988	TENS in human diabetic ulcers	Placebo stimulation: ulcer increased by 28.9 % TENS: 100% healed
Lundeberg *et al.* 1988	TENS in human skin flaps	Placebo stimulation: tissue necrosis in 8/10 patients TENS stimulation: tissue necrosis in 0/14 patients
Lundeberg *et al.* 1992	TENS in human diabetic ulcers	Placebo stimulation: 15% healed TENS: 42% healed

*** In the Brown and Gogia paper, stimulus intensity was set at the minimal voltage required to elicit minimal muscle contraction (i.e. very low intensity TENS).**

PUBLICATION

ABSTRACT

The effects of electroacupuncture (EA) on the healing of full thickness skin incision wounds were examined in the rat. Epidermal wound healing was measured using a non-invasive quantitative method based on the increasing electrical resistance of healing skin. Dermal healing was assessed by measuring the wound breaking strength. We report that EA accelerates epidermal wound healing by 55% and dermal healing by 22% when compared to controls. In addition, EA effects on epidermal wound healing are abolished by nerve block with subcutaneous lidocaine injection. Thus this implicates peripheral nerves in the EA effects. Due to technical reasons the lidocaine blockade could not be tested on dermal effects of EA. This is the first study demonstrating that EA potentiates epidermal and dermal wound healing and that some of this effect is mediated by nerves.

Keywords: electroacupuncture, nerves, wound healing, breaking strength, lidocaine, skin

INTRODUCTION

The beneficial effect of acupuncture on skin wound healing has been described anecdotally for many years (Stux and Pomeranz 1995). Although the acupuncture effects have been described for a long time, the physiological mechanism governing this response has not been fully examined. Previous studies involving electroacupuncture (EA) and wound healing have focused the survival of ischemic skin flaps (Lundeberg *et al.* 1988, Jansen *et al.* 1989a) or ischemic skin ulcers (Kaada 1983). However, more common types of wounds with adequate blood supply such as full thickness skin incisions examined in the present paper, where blood supply is adequate, have not been previously studied.

The current paper addresses the effects of EA on healing of incisional wounds, and the possible role that nerves play in mediating this phenomenon. The role of nerves in normal wound healing has not been well studied. A previous report suggests that denervation impairs the wound contraction phase of wound healing (Engin *et al.* 1996), and our lab has recently shown that complete denervation impairs epidermal wound healing (submitted).

At the turn of the century it was discovered that antidromic stimulation of sensory nerves causes vasodilatation (Bayliss 1901). In later years, this phenomenon has been termed neurogenic inflammation. Neurogenic inflammation is generally thought to be mediated by antidromic release of vasoactive neuropeptides such as: substance P (SP)

and calcitonin gene related peptide (CGRP) (for review see Holzer 1998, Maggi 1991), released from C-fibre afferents. Since inflammation is an important step in wound healing, sensory denervation of the wound should cause less neurogenic inflammation and thus retarded wound healing. This prediction was confirmed when Kjartansson *et al.* (1989) reported that sensory denervation of C-fibres with capsaicin resulted in a decreased survival of skin flaps in rats. Conversely, if nerves were stimulated, neurogenic inflammation could be potentiated and possibly augment the normal healing process. Nerve stimulation either percutaneously by EA or transcutaneously by transcutaneous electrical nerve stimulation (TENS) have been shown to improve healing of ischemic skin flaps (Jansen *et al.* 1989a, Kjartansson *et al.* 1988). This effect was mimicked by injection of CGRP into skin flaps (Kjartansson 1987).

The present study was designed to examine incisional wounds (as opposed to skin flaps) and to test the hypothesis that EA accelerates epidermal and dermal healing. In addition, this study examines the involvement of nerves mediating the EA effects by determining if blockade with the local nerve blocker, lidocaine abolishes the EA effects.

MATERIALS AND METHODS

Adult male Wistar (Charles River) retired breeder rats weighing 350-500 grams were used in the study. The animals were individually housed, given food and water *ad libitum*. In order to reduce stress after delivery, the rats were acclimatized for a week in the animal care facility before experimentation.

Experimental techniques

Surgery

Rats were anesthetized using a cocktail comprised of Atravet (acepromazine maleate, 25 mg/ml), Rogarsetic (ketamine hydrochloride, 100 mg/ml) and Rompun (xylazine hydrochloride, 20 mg/ml) in a 1:7:1. The cocktail was administered intraperitoneally and at a dosage of 1.5 ml/kg body weight. All of the rats received a full thickness experimental wound that consisted of a single midline incision in the interscapular region, orientated rostro-caudally. The wound penetrated the skin and subcutaneous fat down to the panniculus carnosus muscle. The wound was left unsutured and allowed to heal by secondary intention.

Electroacupuncture (EA)

Two pairs of sterile stainless steel acupuncture needles (Ito, Tokyo, Japan) measuring 0.25 mm in diameter and 40 mm in length, were inserted percutaneously, one pair on each side of the incision, 3 cm lateral to the wound. Each pair of needles were 2 mm apart. EA stimulation was delivered through two Grass S48 stimulators using monophasic squarewave pulses 0.2 msec in duration and at 2 Hz. Oscilloscopes were set up to measure the voltage drop over 1 kΩ resistors in series with the needles, and these were used to calculate the amount current being sent to each pair of electrodes. The muscle twitch threshold (MTT) current was established for each pair of needles. The MTT was characterized by the first visible sign of muscle twitching around the needles. A progressive increase in current was given until the MTT was reached. Intensity of EA treatment used for each pair of needles was expressed as a multiple of the amount of current needed to reach MTT for that pair. The MTT in a typical rat was 1 mA, and hence 20xMTT would be 20 mA for that rat.

An EA intensity of 20xMTT was chosen since previous work in ischemic skin flaps use 20 mA with pronounced effects (Jansen *et al.* 1989a). Another group of rats received 5xMTT to test the hypothesis that EA effects are intensity dependent.

EA treatments of 30 minutes were chosen since clinical treatment typically lasts this long. EA was conducted under anesthesia using the cocktail described above.

Measuring Epidermal Healing Using Transcutaneous Electrical Resistance (TER)

A novel method for measuring epidermal wound healing which monitors TER was used to quantify the progress of healing of the incisional wound (Spence and Pomeranz, 1996). This technique is based on the fact that intact skin is relatively impermeable to ions (Edelberg 1967) and therefore has a much higher electrical resistance than wounded skin (Spence and Pomeranz 1996). With healing, the epithelium becomes increasingly impermeable to ions as the ionic barrier is re-established by the stratum corneum (Lykken 1977, Spence and Pomeranz 1996). As the wound heals and the ability to pass ionic current through the wound decreases, Ohm's Law predicts that the resistance would increase proportionately and this was shown by experimentation (Spence and Pomeranz 1996). Skin incisions were made and the progress of wound healing was measured by TER on days 0, 4, 7, 11, and 14 post-wounding for each animal. Animals were shaved prior to surgery and on subsequent testing days. Details of the TER method have been published elsewhere (Spence and Pomeranz 1996). A brief synopsis is given here:

TER was measured using Ag/AgCl non-polarizing electrodes. The recording electrode (length=3.5 cm, diameter=0.1cm) consisted of a Ag/Cl wire encased in a plastic tube (length=3.5 cm, diameter=0.5 cm) filled with 0.9% USP saline. Using a micromanipulator this electrode was gently applied topically in a perpendicular fashion touching the surface of the wound without disrupting the healing process. Hence, this is non-invasive. The electrode was applied for 2 min. each time and was done repeatedly

on days 0, 4, 7, 11, and 14. A cellulose nitrate filter (Satorius SM) covering the bottom of the tube (with 0.1 μm pores) provided a steady supply of saline to the skin surface. The steady stream of saline permeated through the scab and scar tissue and allowed for stable resistance readings within 2 min. of applying the recording electrode to the skin surface.

A reference electrode, a Ag/AgCl plate (length=3.5 cm, width=1 cm, thickness=0.1 cm), was inserted subcutaneously in the dorsal right hindlimb of the rat prior to each recording session. The considerable distance of the reference electrode from the wound was chosen to ensure that the current would pass through the wound, into the body core, and out to the reference electrode (i.e. not over the surface of the wound). Both recording and reference electrodes were connected to a Hioki 3234 Printing Multimeter (Hioki E.E. Corporation, Nagano, Japan) to record DC ohmic resistance.

Resistance measurements were recorded every 30 seconds until stable readings were attained within 2 min. Simple precautions prevented polarization: (a) chloridized electrodes, (b) large electrode surfaces, (c) adequate saline to interface the electrode and tissues. We showed that polarization did not occur since reversal of electrode polarity had no effect on resistance values. In addition, the recording and reference electrodes were short-circuited before every testing session to measure the resistance of the system (including the electrodes) which was less than 2kΩ. Preliminary results have shown that resistance measures taken on day 0 on a freshly cut wound are from 0.3 to 1 kΩ higher

than the short out resistance demonstrating that the resistance of the body core is negligible.

Healing rates were determined from the slopes of semi-logarithmic graph of electrical resistance (kΩ) versus time (days). Linear regression analysis was used to determine the slope for each animal as shown in Fig. 1, which demonstrates results of two typical rats.

Resistance testing was stopped on the day that the resistance surpassed 40 kΩ for the following reasons: 1) Histological studies in our lab show that all wound resistances >40kΩ correlated with intact stratum corneum, which is the final result of epidermal wound healing (Spence and Pomeranz 1996). Thus, a reading of 40 kΩ and one of 1000 kΩ would both represent healed wounds with little difference in their healing. 2) Intact skin, which has never been wounded shows resistances of 40 kΩ or greater, so it is concluded that the healing curve reached completion when 40 kΩ was surpassed. 3) Healed skin would cause plateauing of the healing graphs (see Fig. 1) due to a ceiling effect, which would interfere with the linear regression analysis.

TER has been validated in our laboratory using histology (Spence and Pomeranz 1996), which shows that resistance rises during epidermal healing and that higher resistances were due to re-establishment of the stratum corneum (formed by the keratin deposited by keratinocytes).

Measuring Dermal Healing Using Breaking Strength

In addition to the repeated TERs on each rat described above, wound breaking strength was measured at the end of the experiment on day 15. Breaking strength is a well established method of measuring the wound's ability to withstand tearing (Levenson *et al.* 1965). This differs from tensile strength which incorporates the total cross sectional area of the wound (ideal for homogenous materials). Instead, wound breaking strength measures the strongest layer in a heterogeneous material such as skin. This represents the collagen organization in the dermal level of the skin (Scott *et al.* 1985), and thus is a good measure of dermal healing.

On day 15, rats were euthanized with CO_2 inhalation and had their skin around the wound excised into 4.0 x 0.5 cm wide strips. The breaking strength of these strips was measured by an Instron series 4200 Universal Testing Instrument. The Instron device applied a constant load under computer control at 10mm/min. until the sample tore along the line of the healed wound. The breaking strength in grams was then recorded electronically from the Instron device.

Experiment I – Effects of Repeated EA on Wound Healing

Rats were randomly assigned to either EA or control groups. After the rats were anesthetized (with the cocktail), EA was performed. EA was given for 30 minutes on day

0, 1, 2, and 4 after the experimental wound was made. EA was given at an intensity of 20 or 5xMTT. Control rats received only anaesthetic on day 0, 1, 2, and 4.

TER measurements were taken on day 0, 4 7, 11, and 14. These were done under general anesthesia (using the cocktail). Wound breaking strength measurements were taken on day 15 on euthanized rats.

Experiment II – Effects of Local Anesthetic Nerve Block on EA and Wound Healing

In order to test the hypothesis that EA is mediated by nerves, the muscle twitches were blocked with subcutaneous injection of 2% lidocaine (combined with 1:200,000 epinephrine to reduce systemic absorption of the lidocaine). After establishing the MTT on each rat, 4 ml of lidocaine was administered subcutaneously around the electrodes (2 ml on each side). This dose was shown in pilot experiments to completely block muscle twitches with EA intensities of 20xMTT. Muscle twitches in response to test pulses diminished after a few minutes of injecting the lidocaine. EA intensity was set at 20xMTT as experiment I showed this to be the optimum intensity and EA treatment lasted for 30 minutes. No muscles twitches were observed at 20xMTT because of the lidocaine injection.

Pilot studies showed that repeated lidocaine administration was lethal to some rats. As a result of this, the experimental paradigm was changed to EA treatment and lidocaine being given only once instead of the 4 times in the repeated EA design of

experiment I. Pilot studies also showed that one time EA given on day 2 yielded a greater healing rate than on day 1.

Rats were randomly assigned to one of five groups: 1) EA, 2) EAS, 3) EAL, 4) L, and 5) CON. The EA group underwent EA at 20xMTT for 30 min on day 2. EAS rats underwent EA at 20xMTT for 30 min on day 2 and were concurrently injected subcutaneously around the electrodes with 4 ml with 0.9% USP saline. EAL rats underwent EA at 20xMTT for 30 min on day 2 and were concurrently injected subcutaneously around the electrodes with 4 ml of lidocaine. L rats merely received 4 ml of lidocaine subcutaneously on day 2 (i.e. no EA treatment). CON rats were controls which merely received anaesthetic on day 2 (i.e. no EA or lidocaine).

As in experiment I, TER wound measurements were taken on day 0, 4 7, 11, and 14. Breaking strength measurements were taken on day 15.

As in experiment I, all groups of rats in experiment II, received anaesthetic (cocktail) during EA treatment and TER measurement.

Statistical Analysis

Log resistance values (kΩ) versus time (days) were plotted and linear regression was performed with each rat. The slopes of the regression were used to determine the TER healing rate of each rat. Fig 1 shows 2 typical regression lines for control and

20xMTT. The equation of the control regression is y=0.17x-0.38. In this case the TER slope of this control would be 0.17 (logkΩ)/day. For 20xMTT, the equation of the regression is y=0.33x-0.48 with a slope of 0.33 (logkΩ)/day. The average of TER slopes for each group of rats are expressed as means ± standard error of the mean. One-way ANOVA tests followed by a Bonferroni t-test were used in comparing the TER measurements. This was also done for the mean wound breaking strengths.

RESULTS

Experiment I – Effects of 4 EA sessions and the Influence of Intensity

Visually, the EA rats showed more advanced healing than the control rats. This was not analyzed further as the results were subjective in nature.

EA lead to faster healing and stronger wounds as determined by quantitative objective measures. TER healing rates progressively increased as the intensity of the EA treatment increased. The mean TER wound healing rate for EA at 20xMTT (n=18) and 5xMTT (n=12) were statistically greater than control slopes (n=32) (0.34±0.02 and 0.28±0.01 vs. 0.22±0.01 log (kΩ)/day; $p<0.05$). 20xMTT was also significantly greater than 5xMTT ($p<0.05$). Therefore, 20xMTT results show a 55% acceleration in the rate of wound healing and 5xMTT show a 25% acceleration in the rate of wound healing of at the epidermal level when compared to controls. Fig. 2 summarizes these TER healing rates. Moreover, 20xMTT healing rates were significantly greater than 5xMTT ($p<0.05$).

As shown in fig. 1, linear regression analysis was used to calculate the time required to heal (i.e. to reach a TER of 40 kΩ). As the EA intensity increased, the mean number of days required to heal decreased significantly (Fig. 3). 20xMTT (n=18) and 5xMTT (n=12) rats reached 40 kΩ sooner than control rats (n=32) (6.38±0.32 and 7.68±0.60 vs. 9.89±0.36 days; $p<0.05$). 20xMTT rats also healed significantly sooner than 5xMTT rats ($p<0.05$).

Wound breaking strength was assessed on day 15. The mean wound breaking strength for EA at 20xMTT (n=18) was significantly greater than controls (n=23) (804.0±36.1 vs. 659.1±38.6 grams; $p<0.05$). Thus, there was a 22% increase in the strength of the wound. EA at 5xMTT (n=12) showed no statistical difference in breaking strength when compared to controls. Fig. 4 summarizes these wound breaking strength values.

Experiment II– Effects of One EA Treatment and Influence of Lidocaine

The mean TER wound measurement for EA (single treatment at 20xMTT on day 2) (n=16) was significantly greater than control slopes (n=19) (0.30±0.01 vs. 0.23±0.01 log (kΩ)/day respectively, $p<0.05$). Thus, there was a 30% increase in the rate of epidermal wound healing when EA was given only once. Similarly when looking at the number of days to heal, EA rats healed sooner that control (CON) rats which only received anesthetic (7.41±0.46 vs. 9.49±0.62 days; $p<0.05$). When EA was performed with subcutaneous injections of local anesthetic, lidocaine (EAL) (n=16), the TER healing slope remained at control levels (0.22±0.01 and 0.23±0.01 log (kΩ)/day respectively). Similarly when looking at the number of days to heal, EAL rats healed at approximately the same time as CON rats (9.40±0.55 vs. 9.49±0.62 days; $p<0.05$). These results show that when EA is performed with a local nerve blocker, the epidermal healing rate remained at control levels. When isotonic saline was injected subcutaneously during EA (EAS) (n=15), no significant change in TER slope was seen

when compared to EA by itself (0.32±0.02 vs. 0.29±0.1 log (kΩ)/day respectively). Similarly, EA and EAS took 7.67±0.55 and 7.41±0.46 days to heal respectively.

Wound breaking strength was assessed on day 15. The mean wound breaking strength showed no statistical significant differences between EA and controls suggesting that it was not effective in enhancing dermal repair. Similarly, lidocaine showed no blockade since there was no effect to block.

Comparing 4 EA treatments and 1 EA treatment

In general, the 4 EA treatments was more effective than 1 EA treatment. TER slope values were greater for 4 EA treatments although not statistically significant than 1 EA treatment (0.34±0.02 vs. 0.29±0.01 log (kΩ)/day). Similarly, wounds healed sooner after 4 EA treatments compared to 1 EA treatment (6.38±0.32 vs. 7.41±0.46). However, this effect was not significant. One EA treatment had no significant effect on wound breaking strength whereas 4 EA treatments showed significant effects. Wound breaking strength for 4 EA treatments was not significantly different than 1 EA treatment.

DISCUSSION

This study shows that EA can accelerate epidermal wound healing and increase the wound breaking strength. When given in 4 treatments at 20xMTT EA accelerates epidermal wound healing by 54% or decreases healing time by 3.5 days. It also results in a 22% increase in dermal healing as shown by the wound breaking strength. When EA is given only once (on day 2) at 20xMTT it accelerates wound healing by 30% and decreases healing time by 2 days. However, single EA treatment had no effect on breaking strength. In addition, effects of single acupuncture treatments were blocked with subcutaneous injection of lidocaine. These results demonstrate that EA can accelerate epidermal and dermal wound healing, and that EA effects are mediated by nerves.

Wound healing is a complex process which is generally divided into three phases: inflammation, granulation, and tissue remodeling (Clarke 1996; Daly 1990). Nerves can affect multiple phases in the wound healing process. Neuropeptides play an important role in maintenance of both skin integrity (Brain 1996) and wound healing (for review see Brain 1997). The role of nerves in normal wound healing has not been well studied. Although recent research has shed some light on the neural aspects of wound healing. Engin *et al.* (1996) reported delays in wound contraction after sensory denervation. Recent results in our lab showed that complete peripheral denervation resulted in decreased epidermal healing (submitted). Injection of vasoactive peptides (CGRP) in skin

flaps were shown to increase skin flap survival (Heden *et al.* 1989, Kjartansson *et al.* 1987).

The premise of the present study is that if nerves play a role in normal wound healing, stimulation of nerves may lead to an augmentation of the normal healing process. TENS (Kjartansson *et al.* 1988) and EA (Jansen *et al.* 1989a) and sensory nerve stimulation (Lundeberg *et al.* 1988) have been shown to cause vasodilatation, and thus promote wound healing in ischemic wounds. Jansen *et al.* (1989b) showed that EA and CGRP injection results in similar blood flow increases and suggests that EA blood flow effects are due to CGRP release. EA has also been shown histologically to release SP and CGRP from primary sensory neurons in the rat (Kashiba and Ueda 1991). SP can potentiate inflammation by way of causing vasodilatation and plasma extravasation (Sania 1984). CGRP is also known as a potent vasodilator (Brain *et al.* 1985). These results suggest that EA increases healing of ischemic skin flaps by antidromic stimulation of sensory nerves. However, our incision model does not focus on ischemic wounds, but rather normal healing wounds with a normal blood supply. Hence, other factors other than vasodilatation, may be important in our model. For example increased plasma extravasation could play a role.

Future experiments must address which nerves are responsible for the EA effects. Stimulus intensities of 5xMTT are sufficient for Aδ fibres which can by themselves induce vasodilatation (Jänig and Lisney 1989). In that same study, C-fibre nerve recording showed that they were antidromically stimulated at voltages of 5-15 volts,

which is well in the range of the 20 mA the 20xMTT delivered in our study. Therefore 20xMTT recruits Aδ and C-fibres.

EA working through the potentiation of neurogenic inflammation via the sympathetic nervous system might also account for the EA effects. The sympathetic nervous system has recently been shown to play an important role in neurogenic inflammation (for a review see Basbaum and Levine 1991). Sympathetic postganglionic fibres have been shown to cause potent plasma extravasation in the rat knee joint model (Coderre *et al.* 1989). Recently, our laboratory has found that sympathectomy leads to a decreased wound healing (submitted) and sympathetic stimulation results in acceleration of wound healing (submitted). In addition to direct effects of sympathetics, the sympathetic nervous system can also stimulate nociceptors in inflamed tissue (Sato *et al.* 1993, Sato and Perl 1991, Devor and Jänig 1981) which may contribute to more neurogenic inflammation by antidromic release of SP an CGRP.

The sympathetic nervous system is also reflexively stimulated by somatic afferents. EA (Lin *et al.* 1998, Sato and Schmidt 1966, Sato *et al.* 1996, Karl *et al.* 1975) and somatic afferent nerve stimulation (Blumberg and Wallin 1987, Lundeberg and Norgen 1989) have been shown to stimulate sympathetic spinal cord reflexes to effector organs and to increase blood flow. EA can have biphasic effects on the sympathetic nervous system: EA increases sympathetic nerve activity during stimulation and depresses it afterwards. Yao *et al.* (1982) reported that in spontaneously hypertensive rats, EA stimulation for 30 min caused an initial short lasting elevation in blood pressure

and heart rate, and later, the rats showed depressor responses and bradycardia which lasted 12 hours. Similarly, electrical stimulation of the rat gastrocnemius muscle produced sympathetic stimulation (as seen by elevations in blood pressure and heart rate) while sympathetic inhibition was seen post stimulation. This depressor response lasted over 5 hours (Hoffman *et al.* 1990, Hoffman and Thorén 1988). Dyerhag *et al.* (1997) demonstrated that EA in humans also exerts biphasic effects, during the EA treatment, an increase in skin sympathetic nerve activity was measured, and, 30 minutes after the EA treatment, the skin sympathetic nerve activity decreased. Knardahl *et al.* (1998) showed that EA increased muscle sympathetic nerve activity, with no post-stimulatory effects. In addition, spinal cord stimulation produces peripheral vasodilatation in rats, which appears to be sympathetic in nature (Linderoth *et al.* 1991).

EA has also been shown to reflexively stimulate the parasympathetic system (Oshawa *et al.* 1997). The somatosympathetic reflexes appear to be regulated by opioid system. High dose naloxone (10-15 mg/kg) reversed the cardiovascular depressor response seen in post stimulus EA in rats (Yao *et al.* 1982). Furthermore, low dose κ opioid receptor antagonist, MR 2266 BS blocked the EA post stimulatory blood pressure reduction in rats (Hoffman *et al.* 1990).

Taken together these results may provide a possible mechanism for our observed stimulatory EA effect on wound healing. Firstly, by antidromic stimulation of sensory fibres we are potentiating neurogenic inflammation. Secondly, the sympathetic nervous

system may facilitate healing via somatosympathetic spinal reflexes or direct sympathetic effects. Sympathetic stimulation may further potentiate neurogenic inflammation.

Since EA induces antidromic release of SP (Kashiba and Ueda 1991), direct effects of this peptide may also promote wound healing. Nilsson *et al.* (1985) reported that SP could stimulate DNA synthesis in human skin fibroblasts. This mitogenic effect of SP could potentially play a role in EA wound healing.

We did not use histology in this study and preferred TER to monitor epidermal repair for several reasons: First, in a previous study the TER method was validated by histology (Spence and Pomeranz 1996). Second, problems with histology arose from difficulties encountered in fixation and cutting of rat skin, with the stratum corneum frequently destroyed in the processing of the skin. Hence, differences between experimental and control groups were difficult to show because of these serious artefacts. In contrast, the TER method was used *in vivo* where artefacts were not encountered. Thirdly, TER was a more objective method than histology.

In summary, this is the first study to demonstrate that EA can accelerate epidermal and dermal wound healing in rats, and we have also shown that this phenomenon is a nerve mediated process.

Fig. 1

Typical transcutaneous electrical resistance (TER) healing rates for EA and control rats in experiment I. Intensity of EA treatment is given in multiples of muscle twitch threshold (MTT). Healing rates were calculated from slope of the linear regression equation. 20xMTT (circles) rat has the following regression equation: $y=0.33x-0.48$ ($r^2 = 0.95$) and hence has a healing slope of 0.33 log kΩ/day. Similarly a control rat (triangles) has a linear regression equation: $y=0.17x-0.38$ ($r^2 = 0.93$), and thus has a healing slope of 0.17 log kΩ/day. The horizontal dotted line indicates the healed value (40kΩ or 1.6 logΩ). The vertical dotted line indicates the time required to reach 40 kΩ. A typical 20xMTT and control rat needed 6.34 and 11.51 days to reach 40kΩ respectively.

Fig. 1

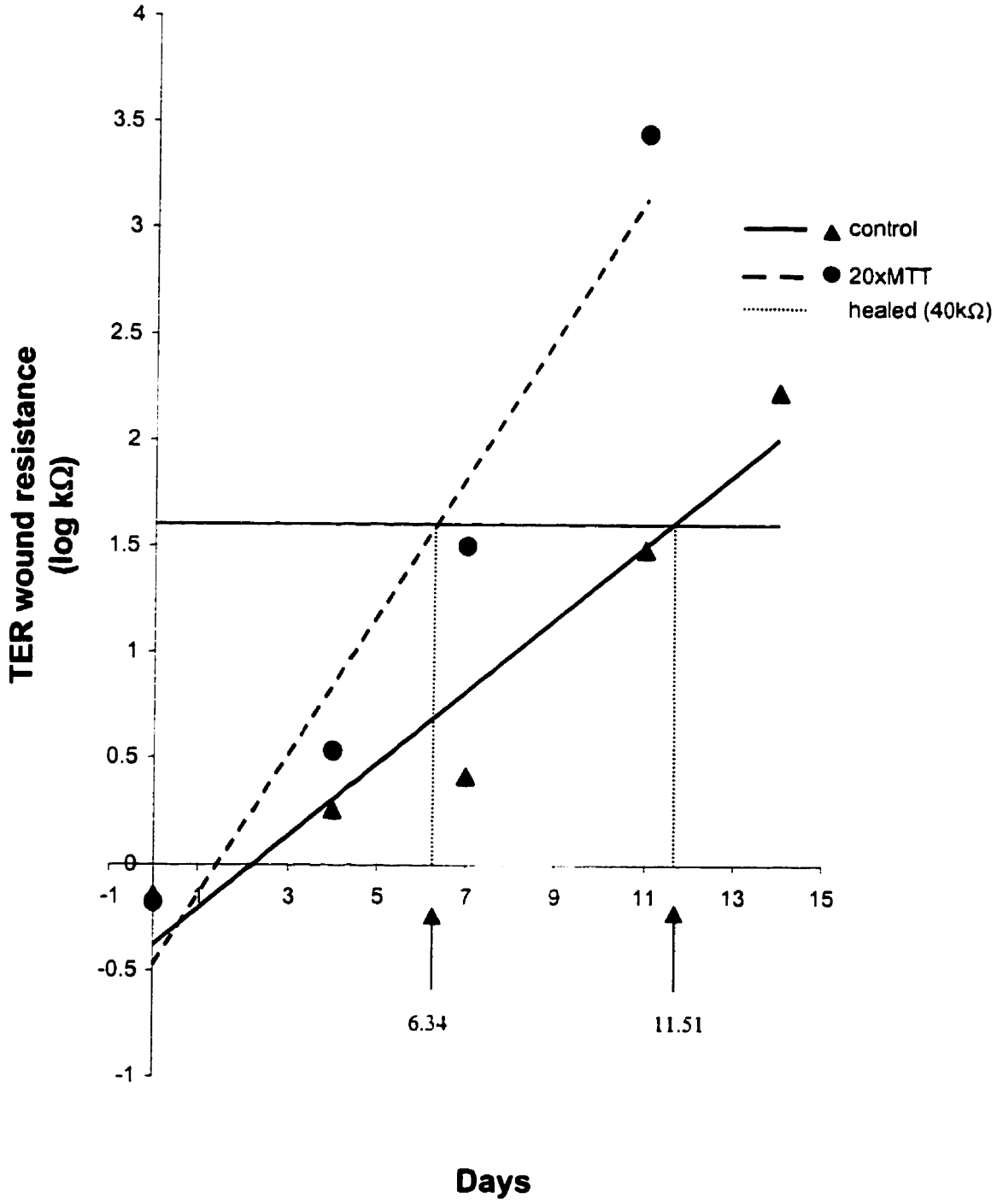

TER wound resistance (log kΩ)
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1
1
3
5
7
9
11
13
15
6.34
11.51
Days
control
20xMTT
healed (40kΩ)

Fig. 2

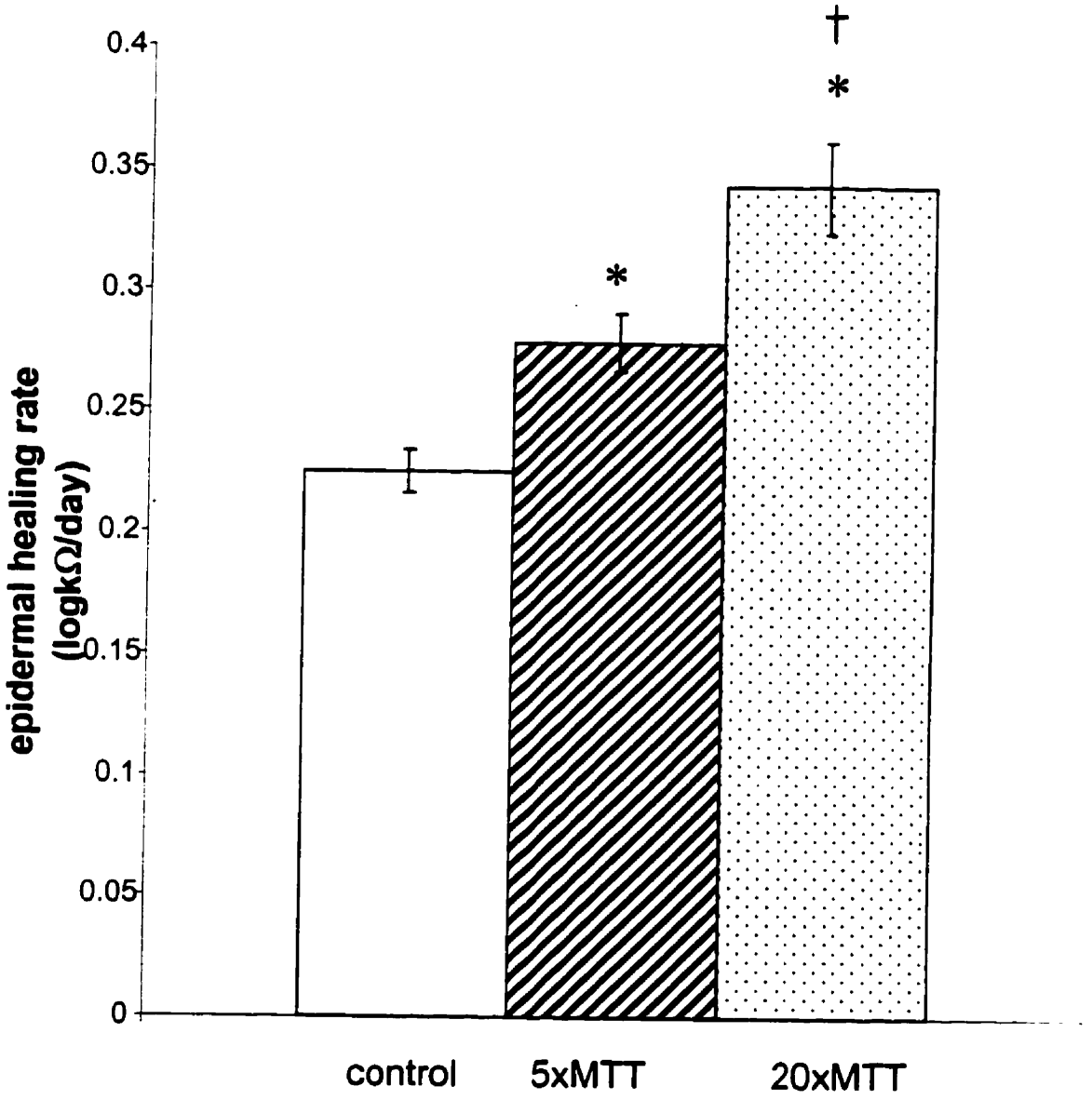

epidermal healing rate
(logkΩ/day)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
*
†
*
control
5xMTT
20xMTT

Fig. 3

Rats heal sooner after 4 electroacupuncture (EA) treatments than controls. Histogram of number of days needed for EA and control rats to reach 40 kΩ. EA intensity is given in multiples of muscle twitch threshold (MTT). 20xMTT and 5xMTT rats healed in 6.38±0.32 and 7.68±0.60 days respectively whereas controls healed in 9.89±0.36 days. Error bars = standard error of the mean. Asterisk indicates a significant difference when compared to controls ($P<0.05$). Dagger indicates that 20xMTT was significantly less than 5xMTT ($P<0.05$).

Fig. 3

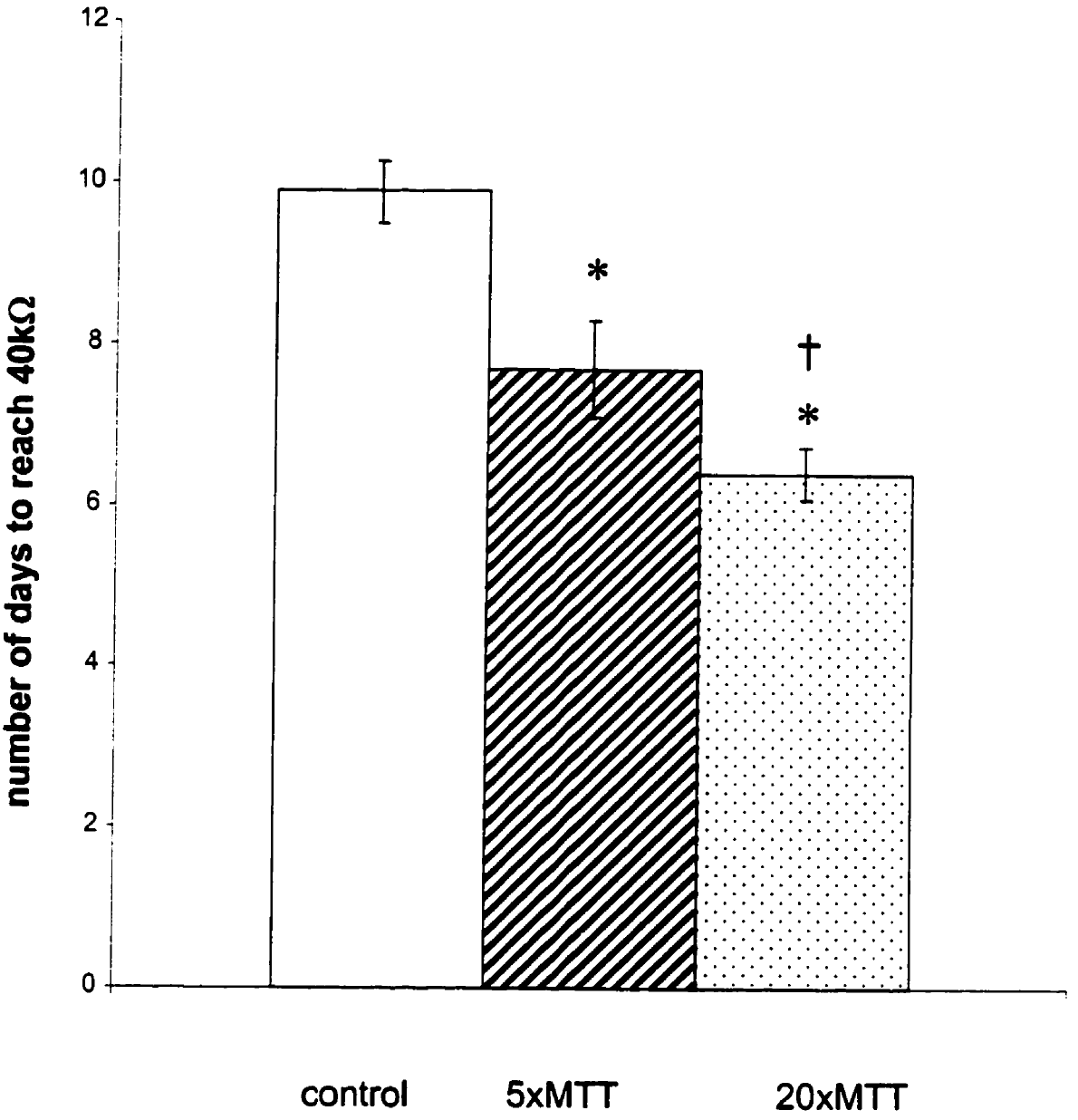

number of days to reach 40kΩ
12
10
8
6
4
2
0
*
†
*
control
5xMTT
20xMTT

Fig. 4

Four treatments of electroacupuncture (EA) potentiate wound breaking strength but at only 20xMTT. 20xMTT and 5xMTT wound breaking strength was 804.0±36.1 and 746.3±40.2 grams respectively. Control wound breaking strength was 659.1±38.6 grams. EA intensity is given in multiples of muscle twitch threshold (MTT). Error bars = standard error of the mean. Asterisk indicates significant difference when compared to controls ($P<0.05$).

Fig. 4

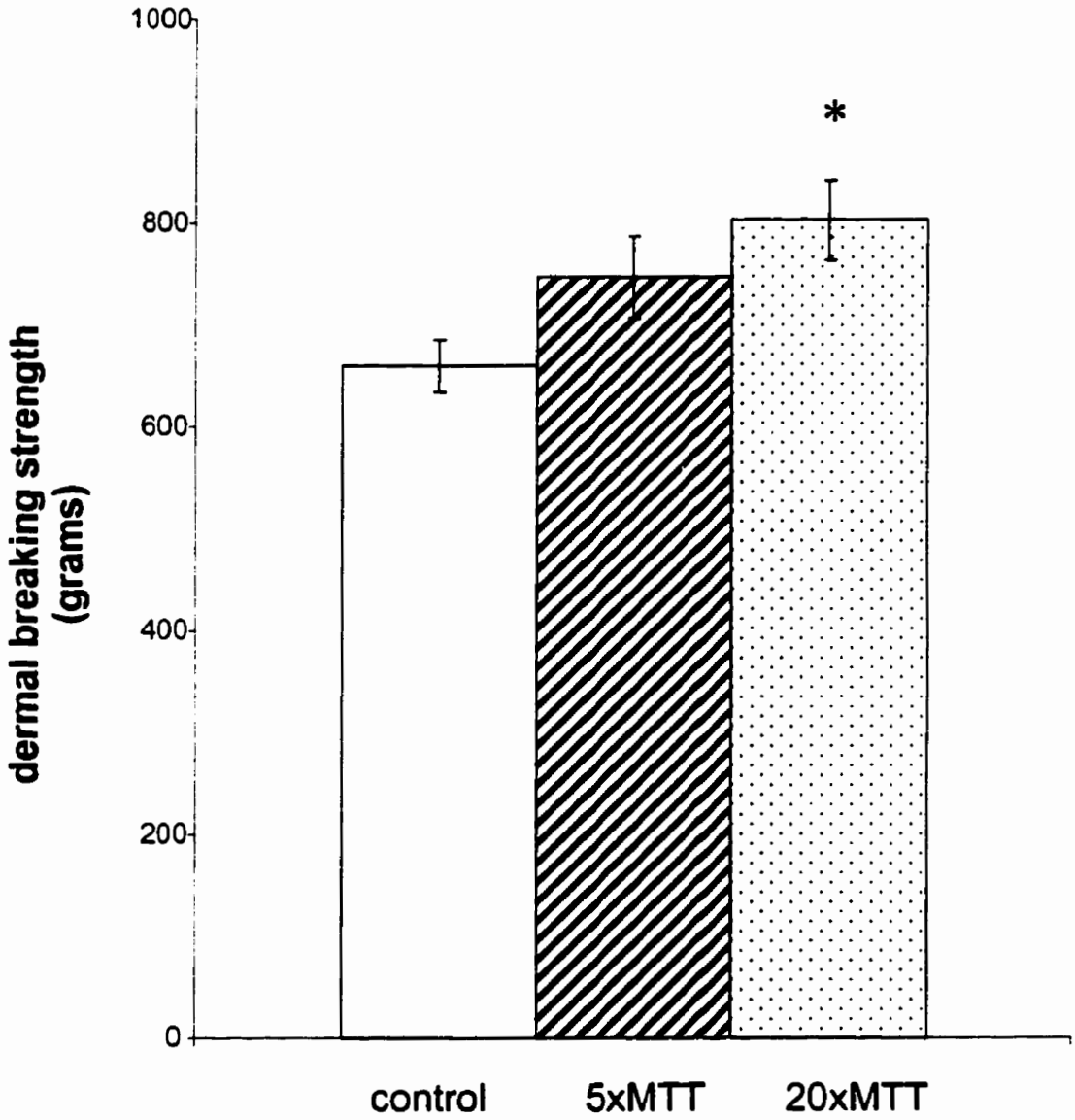

1000
800
600
400
200
0
dermal breaking strength (grams)
*
control
5xMTT
20xMTT

Fig. 5

Histogram of transcutaneous electrical resistance (TER) wound healing. Electroacupuncture (EA) treatment given once potentiates epidermal healing rate; subcutaneous lidocaine injection blocks the EA effects (see bar for EA + lidocaine) since EA + lidocaine is not significantly different than control. Lidocaine has no effect on healing rate (see lidocaine bar) since lidocaine group is not significantly different than control. Error bars = standard error of the mean. Asterisks indicate significant difference when compared to controls ($P<0.05$). Dagger indicates significant difference between EA + saline and EA + lidocaine ($P<0.05$).

Fig. 5

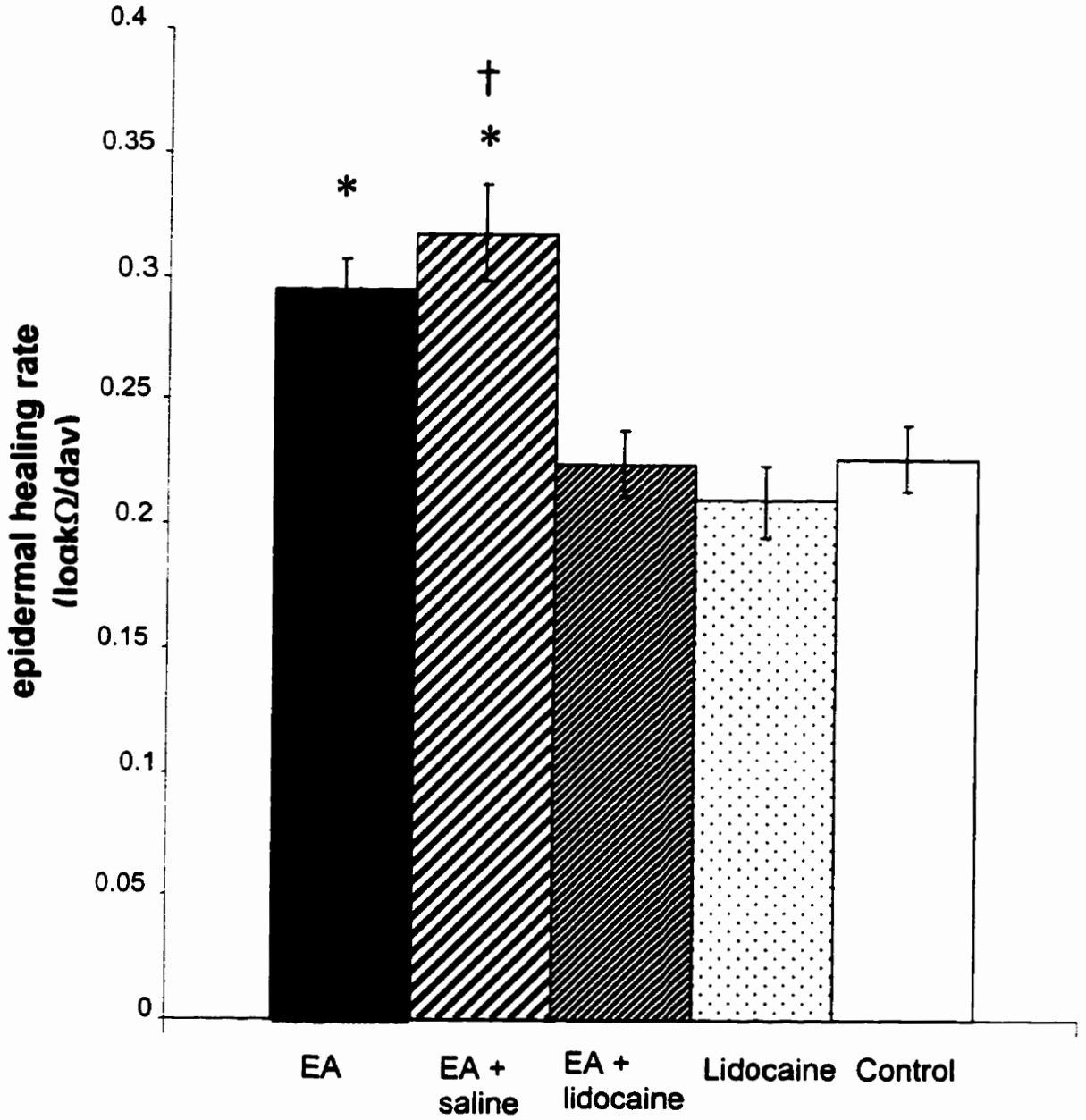

0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
epidermal healing rate
(log kΩ/day)
*
†
*
EA
EA + saline
EA + lidocaine
Lidocaine
Control

Fig. 6

Histogram of the number of days required to reach a TER of 40 kΩ. One treatment of electroacupuncture (EA) causes wounds to heal sooner than controls; subcutaneous lidocaine injection blocks the EA epidermal effects (see EA + lidocaine bar) since EA + lidocaine group is not significantly different than controls. Error bars = standard error of the mean. Asterisks indicate significant difference when compared to controls (anesthetic only) ($P<0.05$). Dagger indicates significant difference between EA + saline and EA + lidocaine ($P<0.05$).

Fig. 6

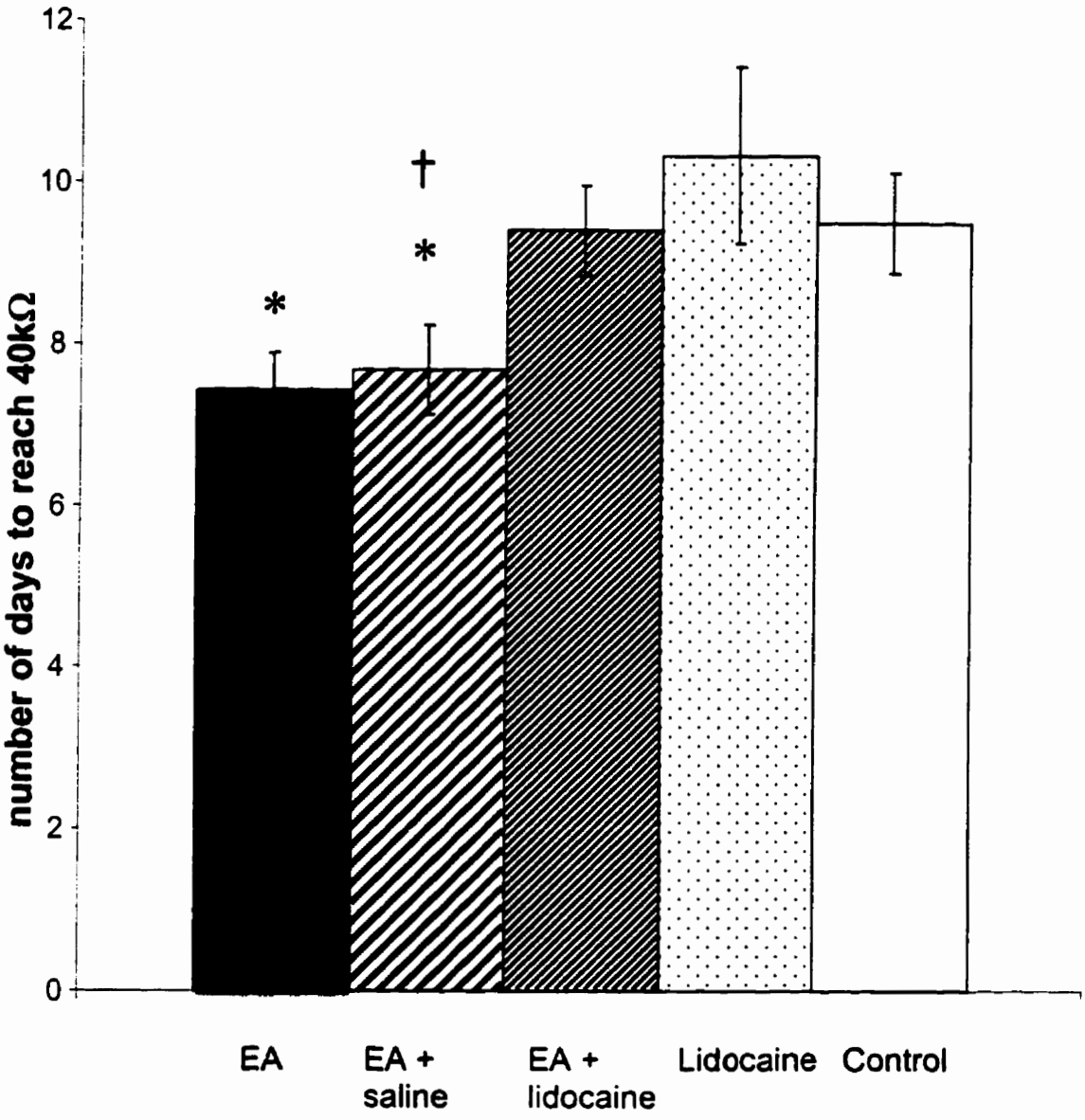
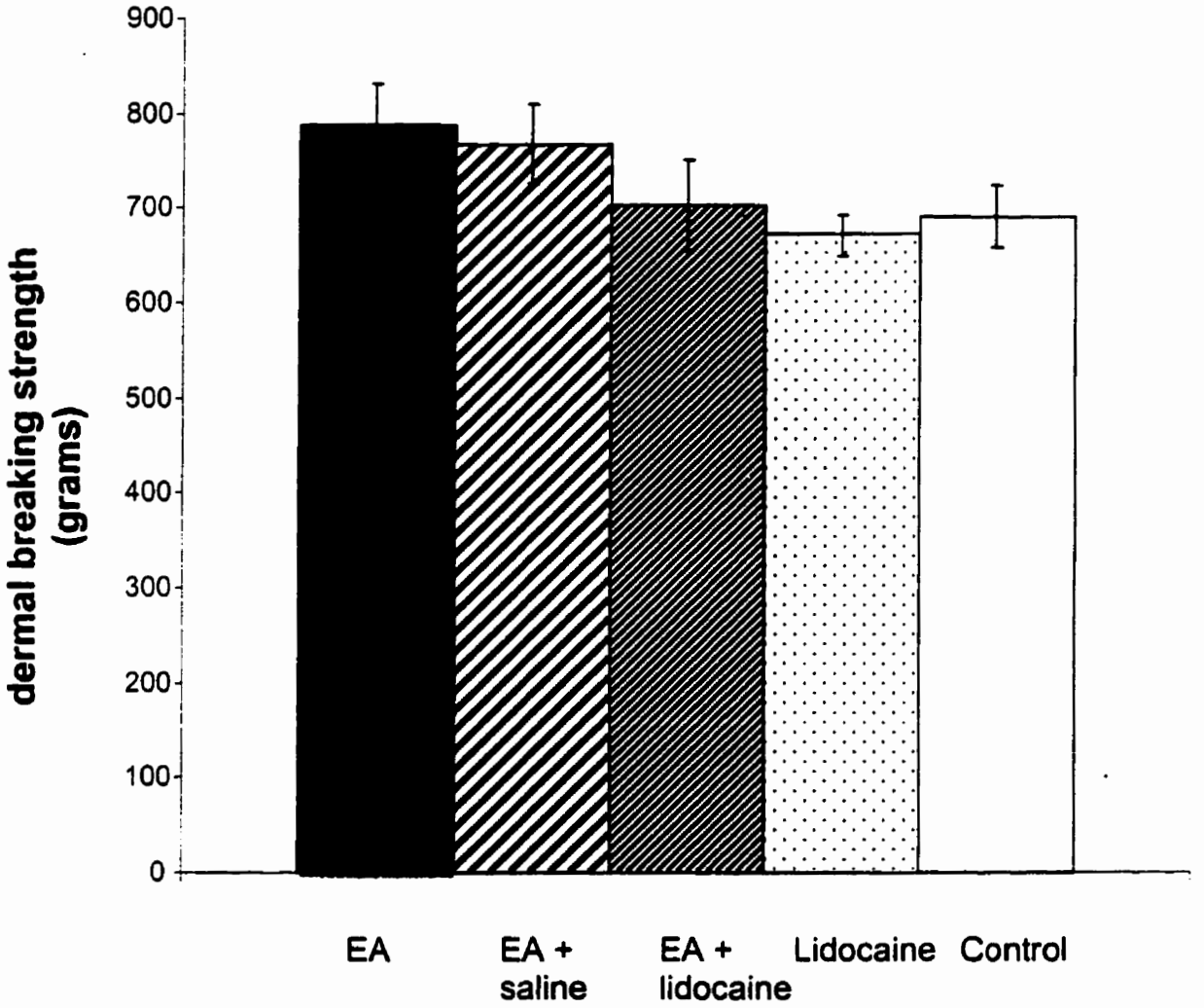

12
10
8
6
4
2
0
number of days to reach 40kΩ
*
†
*
EA
EA + saline
EA + lidocaine
Lidocaine
Control

Fig. 7

One treatment of electroacupuncture (EA) has no significant effect on wound breaking strength. Histogram of the mean of wound breaking strengths after a single EA treatment and effects of lidocaine. Error bars = standard error of the mean. None of the groups were significantly different than the other groups.

Fig. 7


dermal breaking strength (grams)
900
800
700
600
500
400
300
200
100
0
EA
EA + saline
EA + lidocaine
Lidocaine
Control

DISCUSSION

The current study demonstrates that EA given four times can accelerate epidermal and dermal wound healing by 55% and 22% respectively. Single EA treatments accelerate epidermal wound healing by 30%, and this acceleration is blocked by the nerve blocker, lidocaine. This result suggests that the EA effect is mediated by nerves. The results of this thesis confirm other reports that EA and TENS can accelerate healing of ischemic skin flaps (Lundeberg *et al.* 1988, Jansen *et al.* 1989a) or ischemic skin ulcers (Kaada 1983). This discussion section will deal with possible mechanisms for this effect.

Nerves and EA effects

The reversibility of acupuncture effects by way of local anesthetics has been shown in other models. Cheng and Ding (1973) showed that local nerve blocks can abolish acupuncture analgesia. Similarly, using the nerve blocker procaine, Ulett *et al.* (1998) blocked acupuncture analgesia. Dundee and Ghaly (1997) showed that lidocaine injection can block the antiemitic actions of acupuncture.

What nerves are being stimulated?

This study demonstrated that EA effects are mediated by nerves. One major drawback in the study was that no nerve recordings were taken to confirm which nerves are being stimulated during the EA treatment. Evidence from other studies suggest that EA at 20xMTT will stimulate Aα, type II (Aβ) and III (Aδ) and some type IV (C-fibre) neurons. Table 3 summarizes various studies that performed nerve stimulation recorded from the nerves and determined nerve recruitment.

Comparing this study with others is difficult since the stimulus parameters are not always the same. The stimulus pulse can vary in its amplitude and duration. Thus, these 2 parameters must be taken into account when comparing studies. The stimulus threshold of an axon depends on the membrane and internal axon resistance. In general, thick myleninated fibres (Aβ) have a small stimulation threshold since they have a lower internal resistance compared to thin unmyelinated fibres (C-fibre). Although C-fibres have a lower membrane resistance, the high internal resistance results in a high stimulus threshold. C-fibres generally need longer pulse durations compared to thicker myelinated fibres. Thus, the order of fibre recruitment is type II (Aβ; 5 to 10 μm diameter), then type III (Aδ; 1 to 5 μm diameter) then type IV (C-fibre; 0.5 to 1μm diameter). In this study, MTT is usually 1 mA. Since we are measuring resistance over a 1kΩ resister Ohms law (voltage = current x resistance), 20xMTT is typically 20mA or 20V. Kashiba and Ueda (1991) found that EA at 10 V and pulse duration of 0.1 ms caused substantial SP and CRGP release. This suggests that C-fibres were stimulated. Men and Matsui

Table 3 Survey of various studies which examine EA and TENS and consequent nerve type activation.

Study	Type of stimulation	Pulse durations	Nerves involved
Bouhassira *et al* 1986	EA	2 ms	Aδ: 0.25-0.5 mA C: 1-2 mA
Green *et al.* 1995 *	EA	0.25 ms	Aδ: 2.5 mA C: 25 mA
Kashiba and Ueda 1991	EA	0.1 ms	10 V: immunohistochemistry – release of SP and CGRP
Men and Matsui 1994	TENS	0.1 ms	Aβ : 0.219 mA Aδ : 0.413 mA C : 2.1 mA
Okada *et al.* 1996	EA	2 ms	50 mA – supramaximal compound action potential for C-fibres
Toda 1978	EA	0.1 ms	T=threshold for Aβ = 0.053 mA 4xT=Aδ threshold

* Green et al. (1995) did not perform EA, however electrodes were placed percutaneously and stimulated with similar parameters seen in EA.

(1994) showed C-fibre activation at 2.1 mA at a 0.1 ms pulse duration. Green *et al.* (1995) found that stimulating at 25 mA at 0.25 ms duration was a supramaximal intensity to stimulate the all the C-fibres. These studies suggest that EA intensity at 20xMTT in my study was sufficient to recruit the majority of C-fibres but was probably not supramaximal. Based on table 3, EA at 5xMTT is probably sufficient in stimulating type III (Aδ) fibres (Green *et al.* 1995; Mean and Matsui 1994). Other studies involving direct nerve stimulation after dissection have found similar activation thresholds to EA and

TENS. Aδ stimulation intensity was found to be in the range of 0.5 to 3 mA (Janig and Lisney 1989; Oshawa *et al.* 1997; Sato *et al.* 1969; Yao *et al.* 1982). C-fibre stimulation has been found to be in the order of 5 to 44 mA (Clarke et al. 1989; Howheisel and Mense 1990; Kress et al. 1992; McAllister et al. 1995; Wall and Wolf 1984; Wolf and Wall 1986). Further studies using nerve recordings are required to confirm which nerve populations are being stimulated by EA at 20xMTT.

Possible Mechanisms

Some possible mechanisms for the EA effects on wound healing are summarized below. EA stimulation may augment C-fibre neurogenic inflammation. EA may also exert its effect through the sympathetic nervous system.

Potentiating C-fibre Neurogenic Inflammation

As mentioned before, C-fibre nerve stimulation can antidromically release vasoactive peptides. This would increase wound which blood perfusion could promote wound healing by delivering more nutrients, and immune factors to the wound, and at the same time rid the wound of metabolic by products. Antidromic release of SP from primary afferents has been shown to cause vasodilatation and plasma extravasation (Saria 1984). CGRP release from primary afferents is known to cause vasodilatation (Brain *et al.* 1985). Potentiating the affects of SP and CGRP would only occur at stimuli sufficient for C-fibres (20xMTT). At 5xMTT other mechanisms would have to be involved.

Kolston and Lisney (1993) found that stimulating the rat saphenous nerve at Aδ intensity but subthreshold for C-fibre activation caused an increase in blood flow. The mechanism of this Aδ induced vasodilatation has not been worked out. Since the Aδ effects were limited to the ipsilateral paw and there was no change in blood pressure, the authors concluded that the Aδ effects were local and directly related to the stimulation.

Sympathetic Nervous System

The sympathetic nervous system appears to play a role in normal wound healing. Our lab has found that sympathetic stimulation accelerates epidermal healing (submitted) and sympathectomy inhibits wound healing (submitted). The sympathetic nervous system in relation to EA may have 3 effects. Firstly, the sympathetic postganglionic nerve terminal (SPGN) mediates neurogenic inflammation, EA may act through the SPGN to potentiate inflammation and thus wound healing. Secondly, EA during inflammation may potentiate C-fibre neurogenic inflammation through nociceptor sensitization. Thirdly, EA may reflexively inhibit the efferent activity of the sympathetic nervous system. The first 2 effects deal with the proinflammatory aspects of increased local SPGN activity, whereas the latter deals with decreases in efferent nerve activity which may in turn increase blood flow to the wound. These aspects of the sympathetic nervous system will be dealt with below.

SPGN Dependant Neurogenic Inflammation

One of the first studies examining the sympathetic nervous system and inflammation was by Linde *et al.* (1974). In that study, sympathetic stimulation in dogs resulted in a 40% increase in plasma extravastion in adipose tissue. This effect was evident in spite of the pronounced vasoconstriction. In another early study, Engel *et al.* (1978) showed that lumbosacral sympathectomy decreased plasma extravasation. Recent authors have confirmed the importance of the SPGN in neurogenic inflammation (Green *et al.* 1993b; Green *et al.* 1993c; Coderre *et al.* 1989). Coderre *et al.* (1989) showed that rats sympathectomized by chronic 6-hydroxydopamine (6-OHDA) had reduced C-fibre induced plasma extravasation (PE), thus suggesting that C-fibre induced PE is, in part dependant on the SPGN. The SPGN induced PE was found to be dependant on prostaglandins, specifically PGE_2. In addition, the sympathetic nervous system has been shown to produce prostaglandins *in vitro* (Webb *et al.* 1978). PE is reduced when NE is injected in the rat synovial joint (Coderre *et al.* 1989). Thus, there are paradoxical SPGN effects. Namely the SPGN releases NE and neuropeptide Y (NPY) both of which cause vasoconstriction and will reduce PE. Secondly, PGE_2 synthesis by the SPGN will promote PE. Green *et al.* (1993) have proposed a mechanism to explain this paradox. The release of NE and NPY is elicited by orthodromic action potentials and the PGE_2 synthesis will occur from chemical stimulation of the SPGN such as low dose 6-OHDA. In support of this Wakade *et al.* (1991) found that incoming action potentials to the SPGN cause exocytosis from the SPGN via phosphatidylcholine pathway; whereas neurotransmitters acting directly on the SPGN terminals activate the phosphatidylinositol

pathway. There also appears to be interactions between the two pathways. Gonzales *et al.* (1991) found that NE induces prostaglandin production in the SPGN and this is mediated by α-2 adrenergic receptors.

In terms of EA and wound healing, EA might be stimulating the PGE_2 pathway. This will result in a potentiation of inflammation and thus may promote wound healing.

Nociceptor Sensitization Via the Sympathetic Nervous System

It has been known for some time that some types of chronic pain involve the sympathetic nervous system. Pain can often be relieved by sympathetic block (for review see Schwartzman 1987). In addition, patients who have undergone sympatholytic therapy for pain can often have their pain resurface after a subcutaneous injection of norepinephrine (NE) (Torebjörk *et al.* 1995). The nociceptor sensitization seems to occur only during inflammatory conditions, and it is not present in normal undamaged skin. Sato and Kumazawa (1996) demonstrated that in adjuvant-induced arthritis rats, C-fibre polymodal receptors increased their discharge frequency after sympathetic stimulation of NE infusion. The sympathetic induced nociceptor activity work via adrenergic α_2 receptors. The authors suggested that during inflammation there is an upregulation of α_2 receptors. Stimulation of nociceptors via the sympathetic nervous system during inflammation could potentially play a role in wound healing by C-fibre release of CGRP and SP. Since EA stimulation occurred during the inflammatory phase of wound healing, there could have been nociceptor stimulation during EA via somatosympathetic reflexes.

This would inevitably potentiate on going neurogenic inflammation and thus promote wound healing.

Somatosympathetic Reflexes

The effects of EA on the sympathetic nervous system appear to be twofold. In human experiments, sympathetic nerve activity increases during the EA treatment, and in post stimulation there appears to be an inhibition (Dyrehag *et al.* 1997). This appears to represent a similar observation of somatosympathetic reflexes seen in cats. Janig *et al.* (1972) found 75% of cutaneous SPGNs spontaneously active. Stimulating type II and III afferents produced an initial increase in activity followed by a post stimulatory depression. The effects were pronounced when stimulus intensities were sufficient to recruit type IV (C-fibre) afferents. This may explain the human sympathetic nerve activity seen by Dyrehag *et al.* (1997) during and after EA. Moreover, in wound healing, if there is a post stimulatory inhibition of the SPGNs then this could lead to more blood flow to the wound. In addition, the effects are more pronounced when stimulus intensities are sufficient for C-fibres and this intensity dependency was observed in this current study. Also, the post stimulatory inhibition is much longer in duration than the excitation time course (minutes vs. hours), possibly producing more pronounced effects.

Opioids

Opioids can potentially modify wound healing. Yao *et al.* (1982a) showed that stimulation of the sciatic nerve caused a post stimulatory depressor effect which was blocked by naloxone and potentiated by serotonin. Splanchic sympathetic nerve activity showed a post stimulatory depression after similar sciatic nerve stimulation (Yao *et al.* 1982b). The authors implicated central endorphins and serotonin mediating this effect. Also, Hoffman and Thoren (1988) showed similar depressor effects after stimulating the hind leg of spontaneously hypertensive rats. They found that gastrocnemius muscle stimulation for 60 min caused decreases in blood pressure that lasted for over 5 hours. Naloxone injection resulted in a much smaller decrease in blood pressure that lasted only 90 minutes. Blocking 5-HT synthesis abolished the depressor effect. Thus, this implicates endorphinergic and serotinergic systems. This decrease in the sympathetic nervous system activity by removing sympathetic vasoconstriction may promote wound healing through increased regional blood flow to the wound. It appears that opioids are needed to initiate the depressor effects but are not necessarily needed to maintain the depressor effect. Kaada (1982) found that naloxone given many hours after TENS had no effect on skin temperature. Thus he concluded that opioids had no effect on the TENS induced peripheral vasodilatation. The most likely reason there was no effect of naloxne on peripheral vasodilatation was that naloxone was administered after TENS. In a similar experiment, Hoffman and Thoren (1988) injected naloxone prior to the electrical stimulation in the rat and found that the depressor effects were blocked.

Direct Effects of Neurotransmitters

Aside from mediating neurogenic inflammation in EA, neurotransmitters have distinct actions by themselves on wound healing. Some of these independent actions are discussed below.

Substance P

SP and substance K (SK) (another peptide belonging to the tachykinin family found in primary afferents) were found to have mitogenic effects in cultured fibroblasts (Nilsson *et al.* 1985). SP and SK were both found to increase the rate of cells entering S phase. SP also has pronounced effects on the immune system. SP can activate T cells, B cells, monocytes, and granulocytes (for review see Kavelaars *et al.* 1994). Thus, aside from mediating neurogenic inflammation, SP may promote wound healing via direct mitogenic effects and through the immune system.

Calcitonin Gene Related Peptide

CGRP has been found to be co-localized with SP in primary afferent neurons (Hua *et al.* 1987; Lundberg *et al.* 1985). Haegerstrand *et al.* (1990) showed that CGRP stimulated human endothelial cell proliferation and cAMP production in a dose dependant manner. In that same study, other neurotransmitters, neurokinin A (NKA), neuropeptide Y (NPY), and vasoactive intestinal peptide (VIP) had no effects.

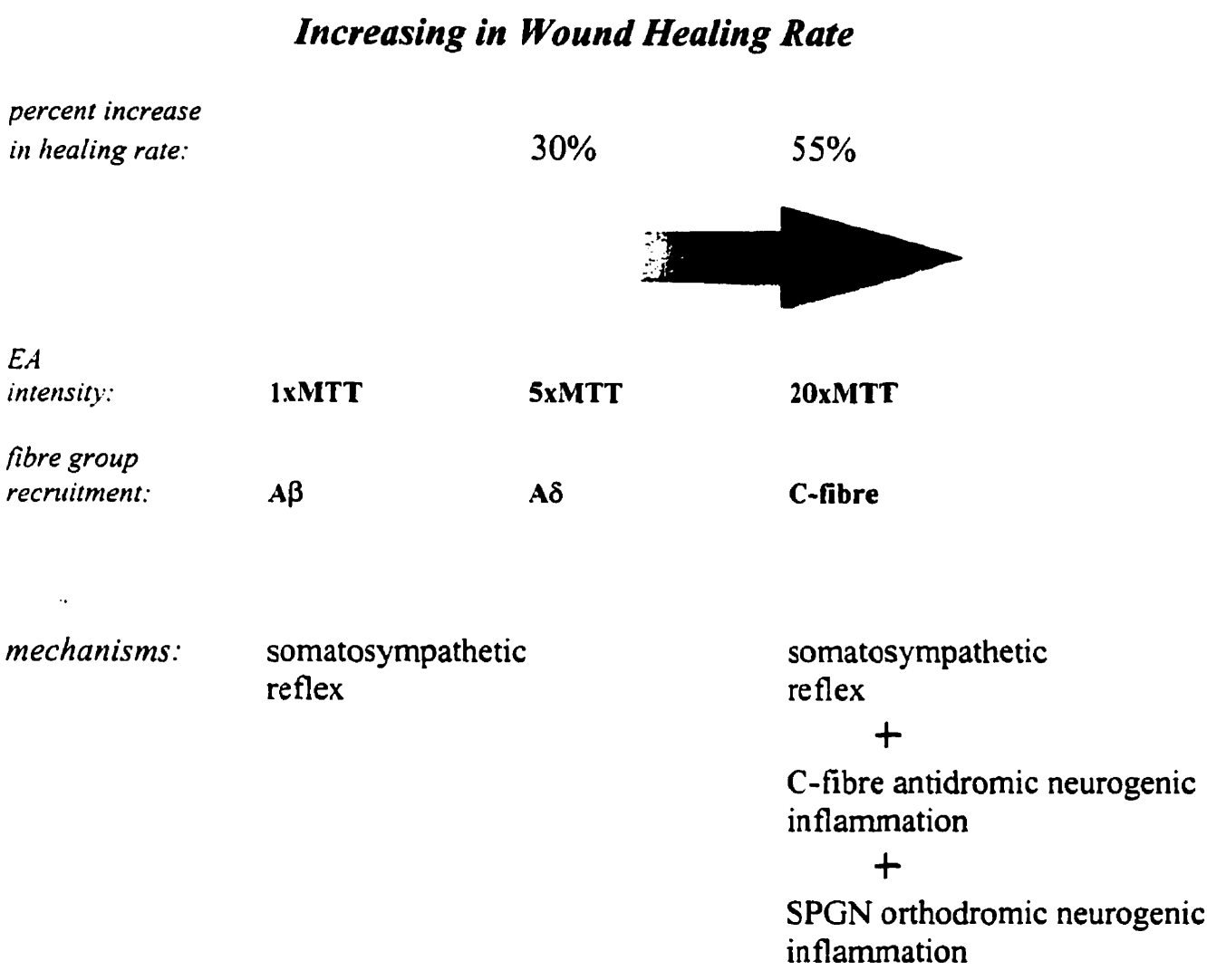
Adenosine

Adenosine, along with NE, NPY, and adenosine triphosphate (ATP) has been found to be present in the SPGN (Schotzinger and Landis 1990). Montesinos *et al.* (1997) found that adenosine A_2 receptors play a role in wound healing. In that study the authors found that topical administration of an A_2 agonist accelerated wound closure in an *in vitro* wound model. Adenosine released from the SPGNs during EA could potentially play a role in accelerating wound healing.

Summary Diagram of Possible EA Mechanisms

Figure 1 summarizes the results obtained by stimulating at 5xMTT and 20xMTT and the possible mechanisms involved.

Figure 1 Proposed mechanism of EA (4 treatments) effects at different intensities. EA intensity is given in multiples of muscle twitch threshold (MTT).



Future Experiments

Future research into EA and its effects on wound healing must fully examine the neural aspects. Experiments involving nerve recording in the nerves to the wound and or the somatosensory cortex must be done to confirm which afferent fibres are being stimulated with the various EA intensities. Also, sympathetic efferent nerve activity during and post EA should be examined. Preliminary studies in our laboratory have shown that distant stimulation in the front paw can affect wound healing of skin in the intrascapular region on the back. This finding suggests that peripheral nerve stimulation can induce systemic effects. The nature of these systemic effects can be examined in several ways. Are the EA effects spinally mediated or supraspinally? This can be examined via intrathecal spinal blocks above the dermatomal level of EA stimulation. What role does the sympathetic nervous system play during EA? Do opiates play a role wound healing? The answers to these questions will help elucidate the mechanism of EA wound healing acceleration. The answer to these questions will also provide clues to what regulates normal wound healing without EA.

The experiments outlined in this thesis showed that EA can accelerate wound healing and the effects are nerve mediated. This opens the door to many more years of research as to how nerves and specifically how their interaction with different physiological systems can mediate healing.

CONCLUSION

This thesis has demonstrated that 4 EA treatments can potentiate epidermal wound healing by 55% and dermal strength by 22%. In order to test the involvement of nerves in this effect, lidocaine was used to block the nerves. Since lidocaine was toxic in repeated doses, only 1 EA treatment was could be tested with lidocaine. One EA treatment accelerates epidermal healing by 30% and this effect was blocked by lidocaine. However, 1 EA treatment produced no effect on dermal healing. Hence, lidocaine blockade of dermal effects of EA could not be demonstrated. Mechanisms involving neurogenic inflammation and the sympathetic nervous system have been proposed. More experiments are needed to elucidate what nerves are being stimulated and the relative involved of primary afferents and SPGNs.

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Fig. A-1

The electroacupuncture schematic is shown. Two pairs of needles are inserted percutaneously 3 cm away from the wound on each side. Each electrode pair forms a closed circuit in which the current (square pulse 0.2 msec in duration) of the system is monitored through an oscilloscope over a 1 kΩ resistor. The output of the two stimulators are trigged by an external stimulator to allow for synchronous stimulations on both sides.

APPENDIX A

EXPERIMENTAL SETUP

Fig. A-1

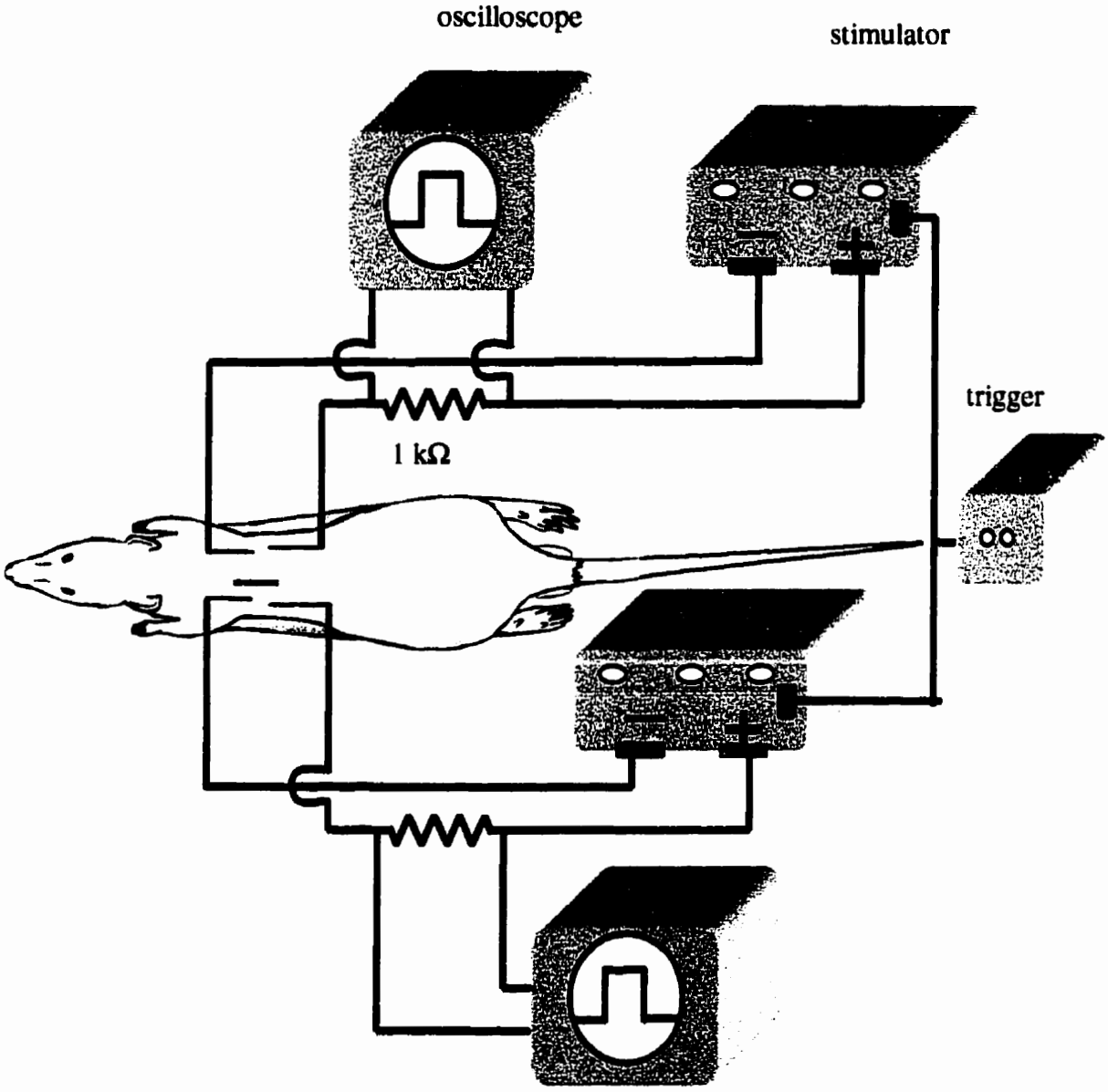


Fig. A-2

A)

The transcutaneous electrical resistance (TER) apparatus is shown. The experimental wound is made in the mid-scapular region. The active electrode is placed on the surface of the wound, while the indifferent electrode is inserted subcutaneously in the thigh region. The resistance between both electrodes is measured using the Hioki ohmmeter.

B)

A detailed schematic of the above is shown. The current flow through the TER system is shown.

Fig. A-2

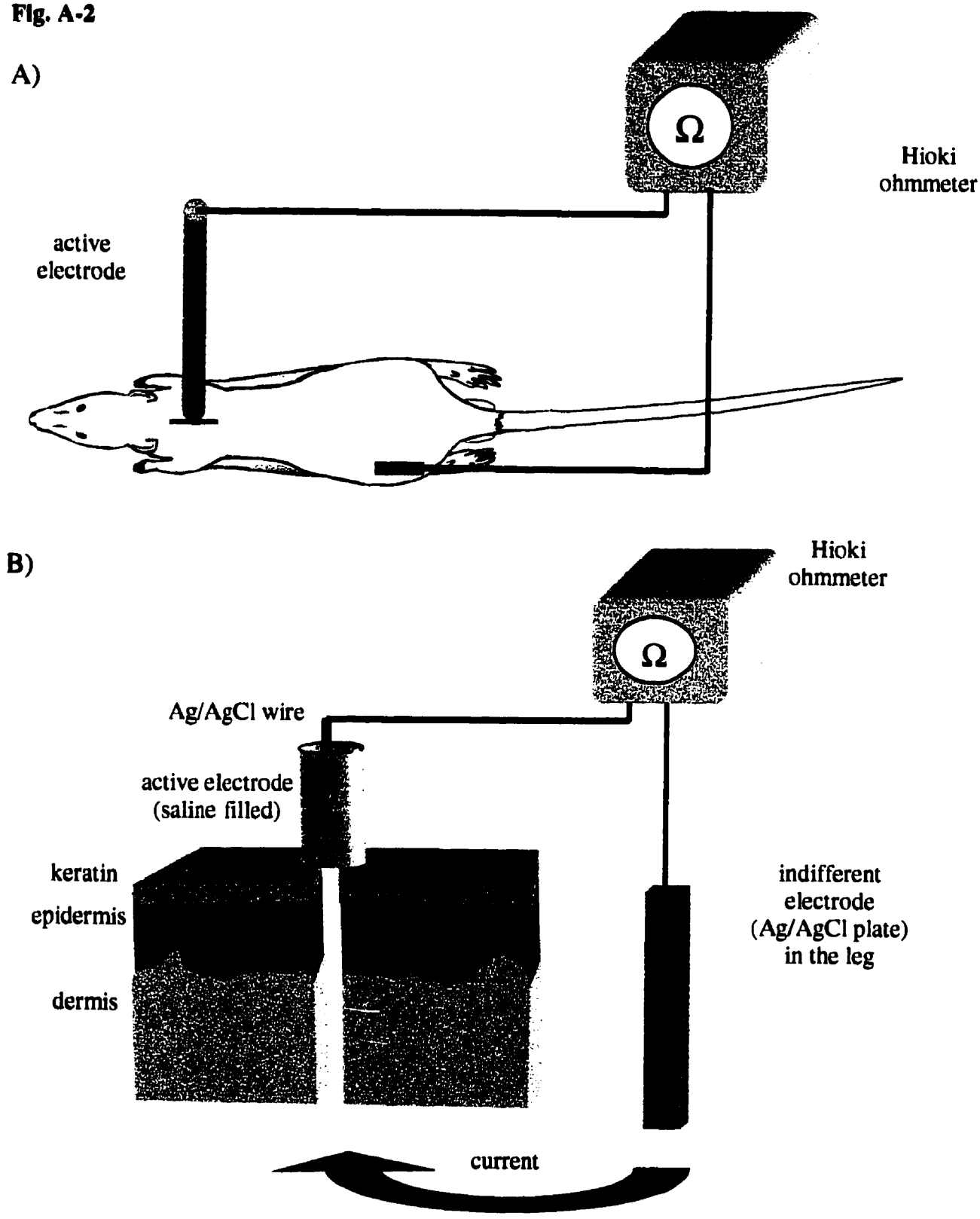

A)
Ω
Hioki
ohmmeter
active
electrode
B)
Hioki
ohmmeter
Ω
Ag/AgCl wire
active electrode
(saline filled)
keratin
epidermis
dermis
indifferent
electrode
(Ag/AgCl plate)
in the leg
current

Fig. A-3

The Instron universal testing instrument is shown (Universal 4200). The skin sample is clamped in the instrument while the mobile crossbeam under computer control is raised at 10 mm/min. The computer displays the peak force required to break the tissue (in grams).

Fig. A-3

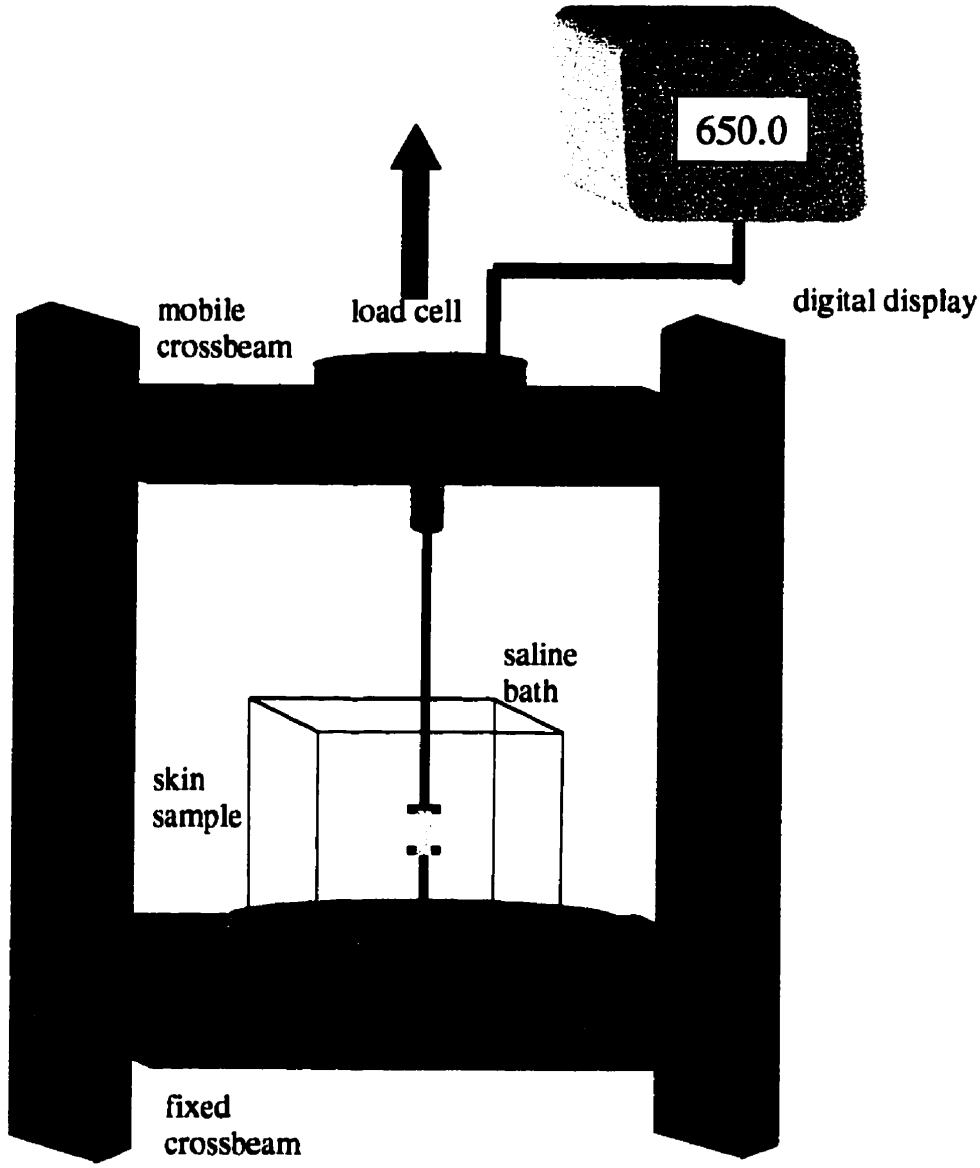

650.0
digital display
load cell
mobile
crossbeam
saline
bath
skin
sample
fixed
crossbeam

Fig. B-1

A)

Photograph of a typical control rat wound 7 days post-operatively. Scale = 1cm

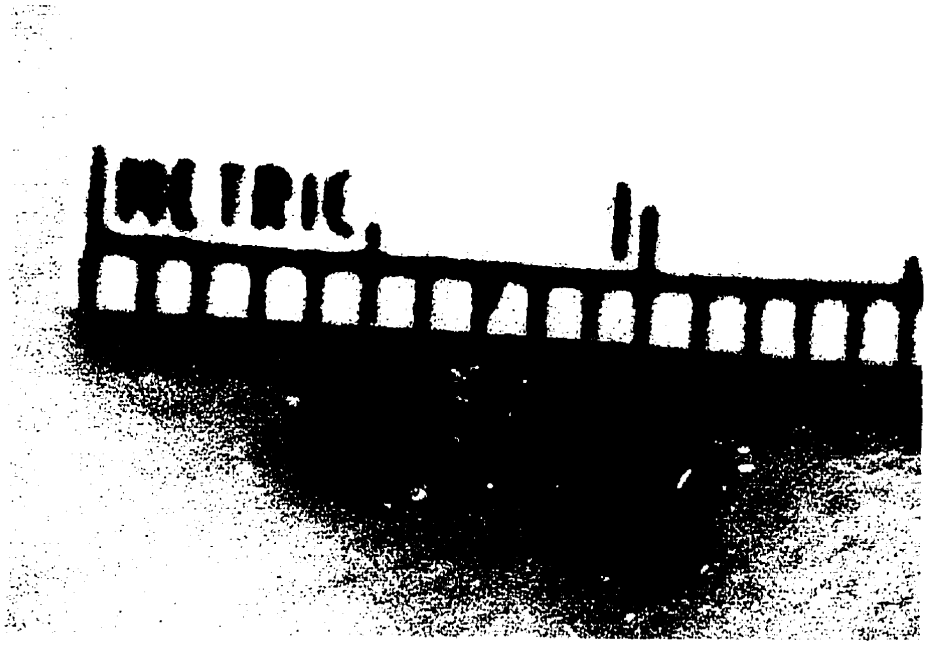
B)

Photograph of a typical electroacupuncture rat wound 7 days post-operatively. Scale = 1cm

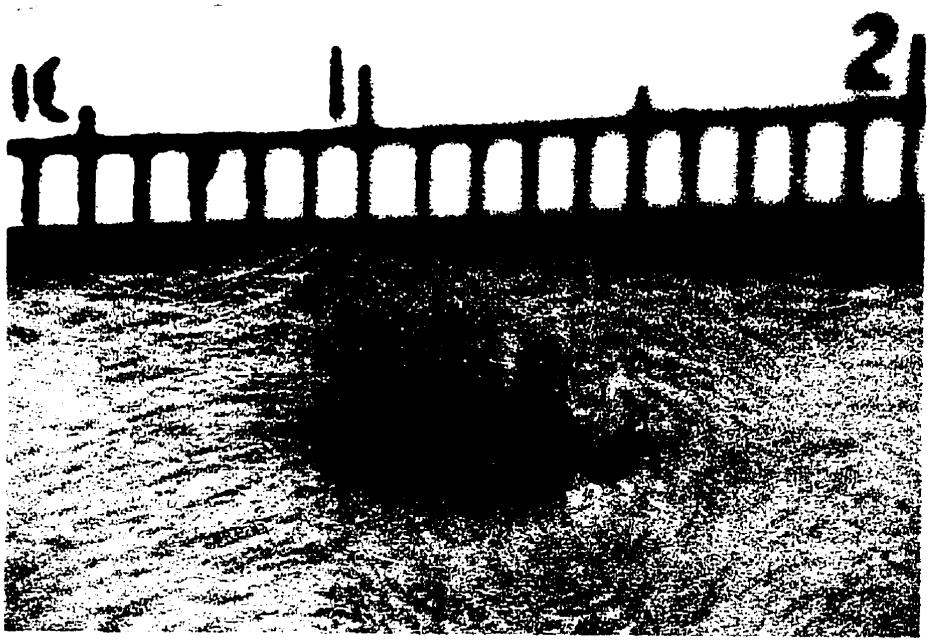
APPENDIX B

Fig. B-1

A)



B)



APPENDIX C

Table C-1 Healing slopes (log kΩ/day) for each rat are shown. Electroacupuncture (EA) intensity is given in multiples of threshold current needed to elicit muscle twitch (MTT). Rats received 4 EA treatments.

	control		5xMTT		20xMTT	
	slope	r^2	slope	r^2	slope	r^2
	0.139	0.943	0.271	0.854	0.353	0.871
	0.267	0.864	0.292	0.811	0.391	0.901
	0.244	0.878	0.330	0.875	0.298	0.901
	0.194	0.892	0.181	0.939	0.414	0.998
	0.264	0.836	0.315	0.813	0.340	0.807
	0.278	0.878	0.292	0.861	0.329	0.949
	0.307	0.869	0.238	0.791	0.636	1.000
	0.185	0.984	0.297	0.852	0.323	0.942
	0.239	0.829	0.235	0.912	0.265	0.942
	0.167	0.921	0.292	0.810	0.343	0.851
	0.288	0.873	0.280	0.918	0.335	0.820
	0.195	0.871	0.310	0.851	0.271	0.952
	0.264	0.749			0.339	0.773
	0.176	0.926			0.316	0.773
	0.218	0.858			0.257	0.840
	0.224	0.993			0.260	0.986
	0.184	0.935			0.389	0.865
	0.207	0.774			0.306	0.818
	0.200	0.812				
	0.218	0.903				
	0.253	0.797				
	0.170	0.932				
	0.231	0.722				
	0.136	0.781				
	0.240	0.750				
	0.323	0.775				
	0.242	0.891				
	0.319	0.719				
	0.221	0.775				
	0.249	0.789				
	0.180	0.820				
	0.161	0.942				
average	0.224	0.878	0.278	0.857	0.343	0.888
SD	0.049	0.074	0.041	0.047	0.086	0.075
SEM	0.009	0.013	0.012	0.014	0.020	0.018
n	32	32	12	12	18	18

Table C-2 Breaking strengths (grams) for each rat are shown. Electroacupuncture (EA) intensity is given in multiples of threshold current needed to elicit muscle twitch (MTT). Rats received 4 EA treatments.

	control	5xMTT	20xMTT
	627.1	536.1	918.1
	526.0	490.0	701.6
	833.3	536.9	807.6
	578.1	827.4	1062.0
	717.7	815.0	982.6
	779.6	1081.0	1073.0
	585.9	683.0	851.0
	831.7	826.0	790.3
	459.0	659.0	603.1
	600.1	864.0	595.0
	697.8	829.5	710.9
	443.9	795.3	781.2
	580.2		774.8
	832.2		1061.0
	620.5		601.8
	633.3		597.9
	849.4		917.6
	800.5		641.7
	649.0		
	564.4		
	680.2		
	509.6		
	760.8		
average	665.5	746.8	804.0
SD	128.0	155.6	153.2
SEM	38.6	40.2	36.1
n	19	12	18

Table C-3 Number of days to heal for each rat are shown. Electroacupuncture intensity is given in multiples of threshold current needed to elicit muscle twitch (MTT). Rats received 4 EA treatments.

	control	5xMTT	20xMTT
	12.536	8.858	6.764
	9.029	8.303	4.255
	8.821	3.960	8.030
	11.830	11.760	5.933
	9.008	8.237	6.949
	7.807	8.152	6.337
	8.091	4.370	3.081
	12.083	7.703	5.212
	9.324	9.100	8.061
	12.168	7.856	6.778
	9.415	7.162	7.208
	10.062	6.707	7.825
	9.728		5.266
	12.394		6.198
	11.108		7.353
	5.406		6.929
	11.644		5.725
	11.105		6.947
	7.933		
	7.933		
	9.728		
	9.096		
	11.628		
	11.987		
	11.223		
	8.432		
	7.622		
	10.691		
	3.745		
	10.786		
average	9.888	7.681	6.381
SD	2.053	2.067	1.536
SEM	0.362	0.597	0.362
n	32	12	18

Table C-3 Number of days to heal for each rat are shown. Electroacupuncture intensity is given in multiples of threshold current needed to elicit muscle twitch (MTT). Rats received 4 EA treatments.

	control	5xMTT	20xMTT
	12.536	8.858	6.764
	9.029	8.303	4.255
	8.821	3.96	8.03
	11.83	11.76	5.933
	9.008	8.237	6.949
	7.807	8.152	6.337
	8.091	4.37	3.081
	12.083	7.703	5.212
	9.324	9.1	8.061
	12.168	7.856	6.778
	9.415	7.162	7.208
	10.062	6.707	7.825
	9.728		5.266
	12.394		6.198
	11.108		7.353
	5.406		6.929
	11.644		5.725
	11.105		6.947
	7.933		
	7.933		
	9.728		
	9.096		
	11.628		
	11.987		
	11.223		
	8.432		
	7.622		
	10.691		
	3.745		
	10.786		
average	9.888	7.681	6.381
SD	2.053	2.067	1.536
SEM	0.362	0.597	0.362
n	32	12	18

Table C-4 Healing slopes (log kΩ/day) for each rat are shown. EA: electroacupuncture, EAS: electroacupuncture + saline, EAL: electroacupuncture + lidocaine, L: lidocaine by itself, CON: anesthetic by itself. Rats received only 1 EA treatment.

	EA		EAS		EAL		L		CON	
	slope	r^2	slope	r^2	slope	r^2	slope	r^2	slope	r^2
	0.301	0.868	0.371	0.804	0.275	0.892	0.211	0.97	0.222	0.985
	0.296	0.989	0.328	0.919	0.216	0.813	0.199	0.964	0.212	0.986
	0.272	0.978	0.34	0.963	0.297	0.911	0.164	0.704	0.215	0.875
	0.267	0.983	0.366	0.976	0.278	0.857	0.169	0.938	0.22	0.82
	0.392	0.863	0.357	0.935	0.265	0.991	0.19	0.915	0.231	0.722
	0.284	0.797	0.204	0.951	0.203	0.953	0.119	0.702	0.136	0.781
	0.272	0.996	0.27	0.919	0.158	0.967	0.146	0.841	0.24	0.75
	0.344	0.975	0.185	0.672	0.168	0.929	0.294	0.925	0.323	0.775
	0.271	0.786	0.311	0.914	0.185	0.957	0.201	0.923	0.242	0.891
	0.246	0.804	0.305	0.975	0.18	0.908	0.111	0.923	0.319	0.719
	0.361	0.942	0.37	0.834	0.264	0.876	0.217	0.965	0.221	0.775
	0.251	0.752	0.441	0.878	0.24	0.925	0.398	0.879	0.249	0.789
	0.332	0.887	0.349	0.997	0.149	0.923	0.276	0.86	0.18	0.82
	0.343	0.95	0.197	0.798	0.262	0.968	0.238	0.894	0.161	0.942
	0.249	0.875	0.368	0.904	0.222	0.829			0.237	0.93
	0.239	0.743							0.262	0.925
									0.265	0.924
									0.139	0.967
									0.24	0.81
average	0.295	0.887	0.317	0.896	0.224	0.913	0.21	0.886	0.227	0.852
SD	0.046	0.089	0.074	0.087	0.048	0.052	0.076	0.086	0.05	0.09
SEM	0.012	0.022	0.019	0.022	0.013	0.013	0.02	0.023	0.011	0.021
n	16	16	15	15	15	15	14	14	19	19

Table C-5 Wound breaking strength (grams) for each rat are shown. EA: electroacupuncture, EAS: electroacupuncture + saline, EAL: electroacupuncture + lidocaine, L: lidocaine by itself, CON: anesthetic by itself. Rats received only 1 EA treatment.

	EA	EAS	EAL	L	CON
	419	725	683.6	809.1	549.8
	539.8	1007	1153	720.6	537.1
	852.1	994.4	863.9	644.4	720.6
	794.1	835.4	693.9	599.2	516.7
	574.9	663.2	932.6	807.4	443.9
	812.3	594.7	585	644.7	580.2
	844	785.2	619.5	596.5	832.2
	842.4	662.9	550.1	634.9	620.5
	844.6	702.1	711.8	650.9	633.3
	664.5	586.1	635.4	570.6	849.4
	944.4	737.1	660.1	619.6	800.5
	1066	863.4	652.2	590.8	649
	801.1	599.1	597.2	920.1	702.7
	1159	993.8	660.4	594.9	904.5
	754		553.7		774.5
	745.5				693.7
	732.8				920.9
average	787.7	767.8	703.5	671.7	690
SD	179.9	150.4	162.6	104.1	141.6
SEM	43.6	41.1	48.3	21.8	32.5
n	17	14	15	14	17