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# Acupuncture in a neuroimmunological perspective

Peripheral effects

# Bacterial challenge

## from inflammation to pain

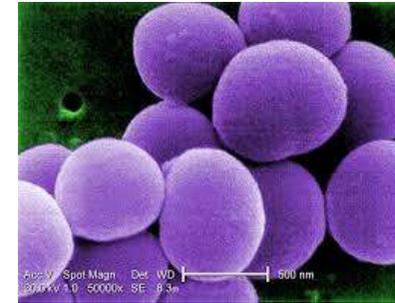
Bacterial infection induces inflammation through immune cell recruitment.

Inflammatory pain during infection is triggered by the action of

- immune-derived proteins (e.g. cytokines and growth factors),
- lipids (e.g. prostaglandins)
- amines
- potassium
- protons

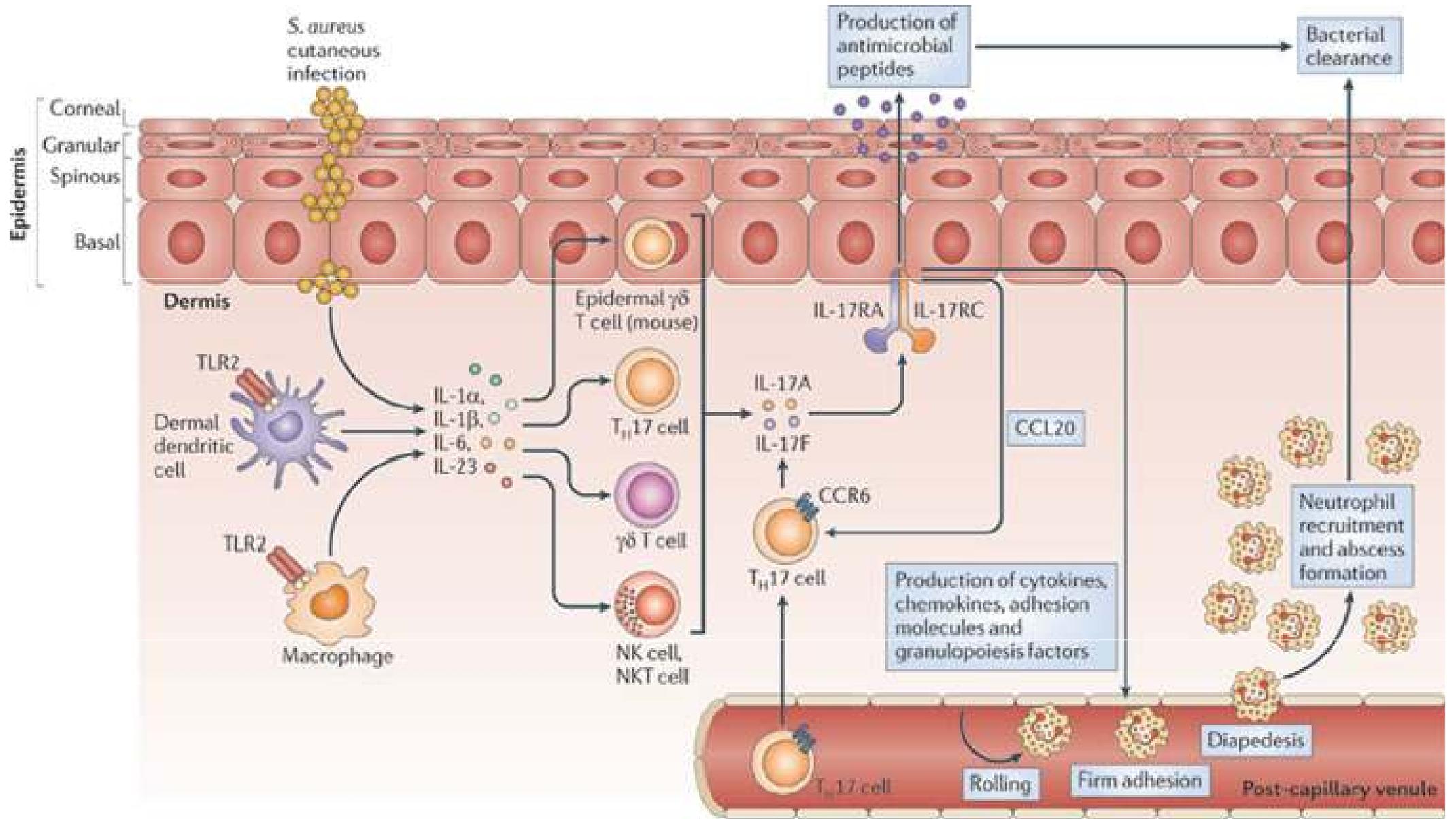
on receptors expressed by nociceptors

# *S. aureus*



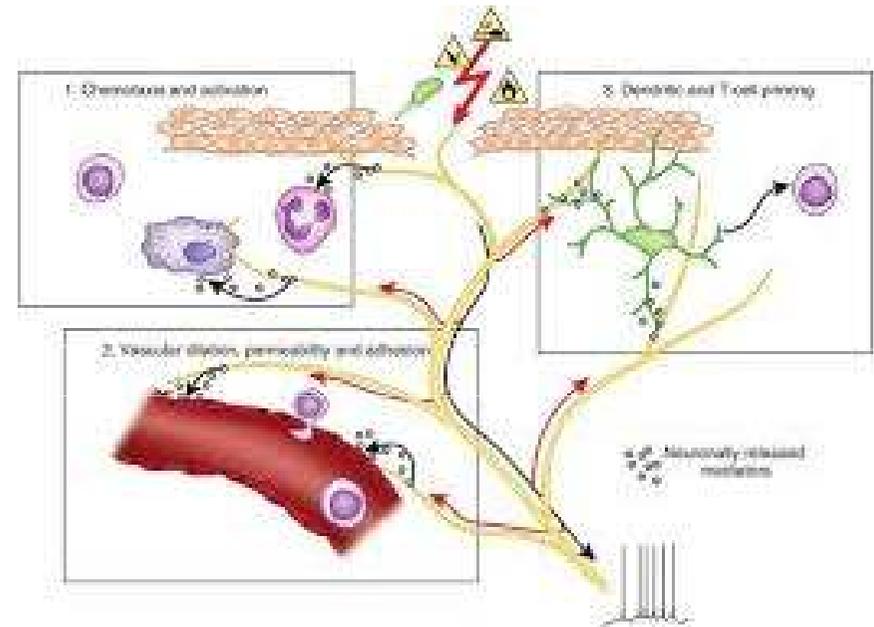
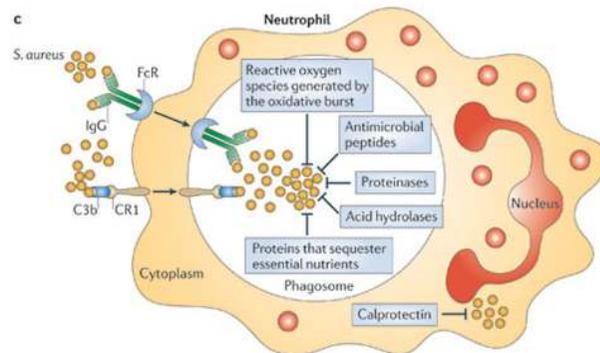
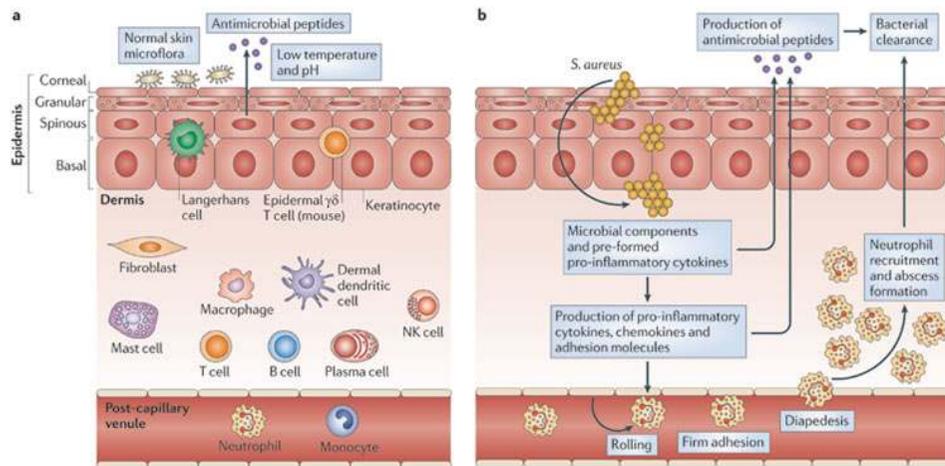
*S. aureus* is a major cause of wound and surgical infections, leading to painful abscesses, cellulitis, necrotizing fasciitis.

*S. aureus* releases toxins including hemolysins, Pantone- Valentine Leukocidin (PVL), and phenol soluble modulins, which play roles in bacterial dissemination and tissue damage.



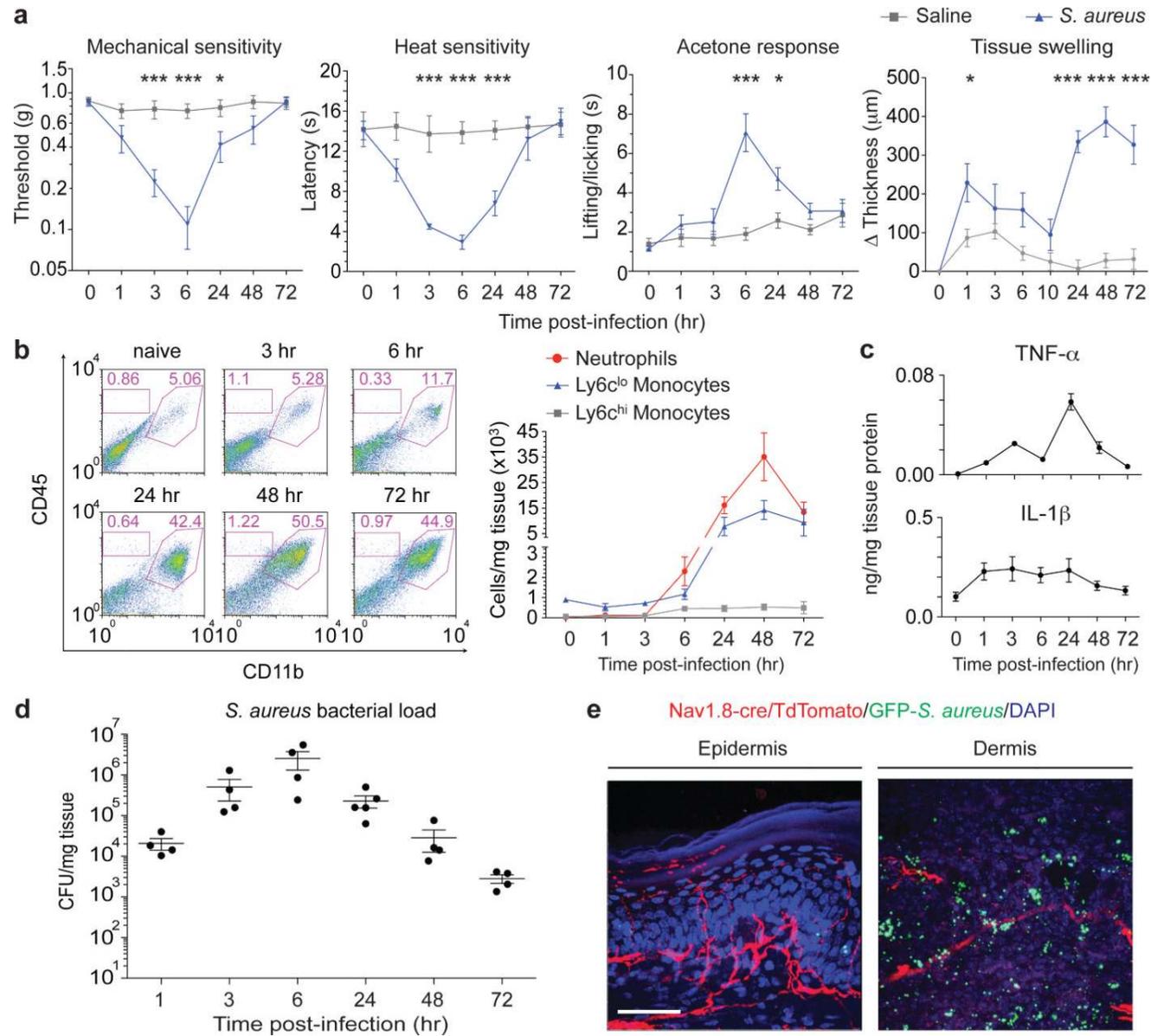
# Challenging the concept

Woolf and collaborators (*Nature* 2014)



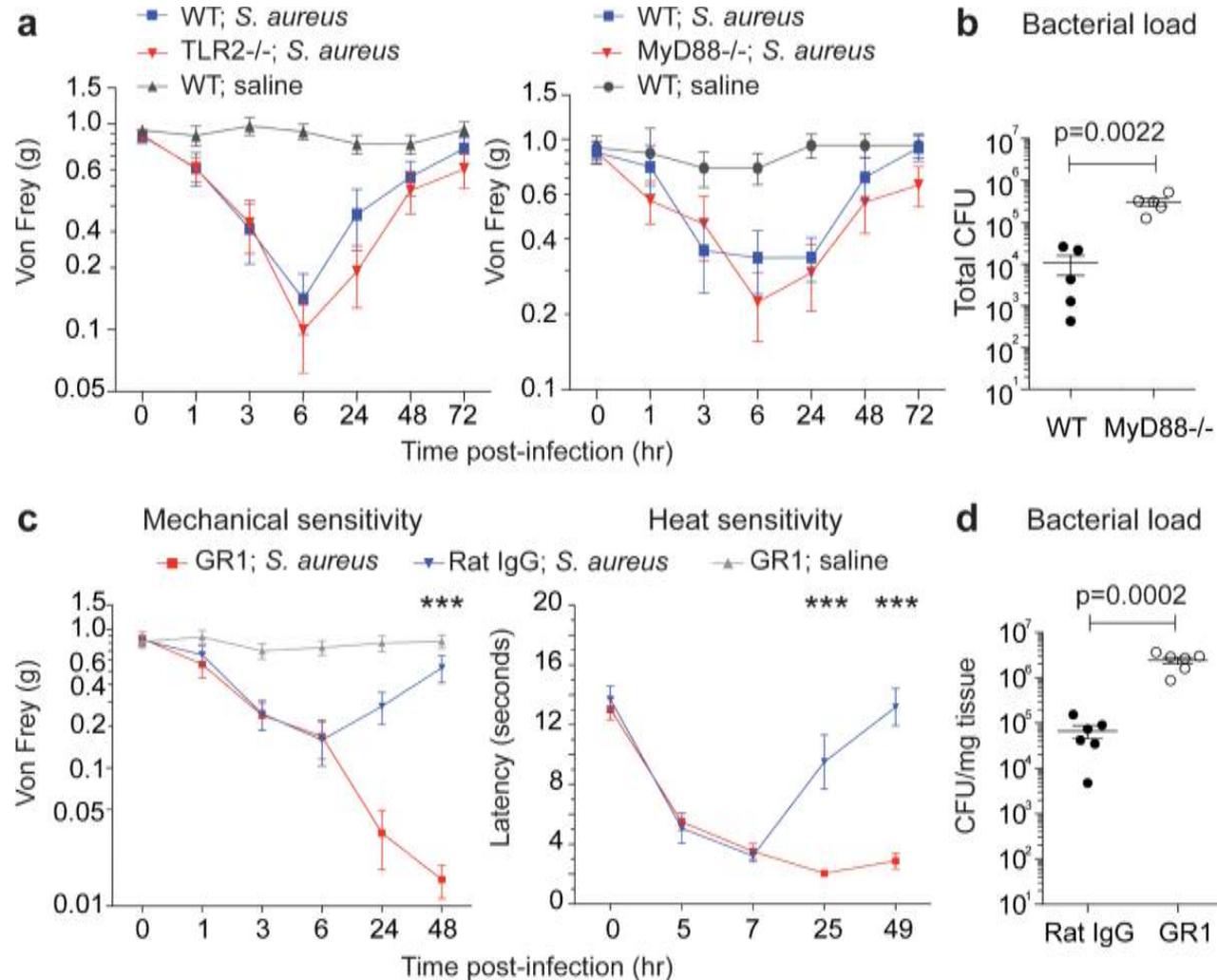
**Pain correlates with bacterial load**

# *S. aureus* infection induces pain hypersensitivity paralleling bacterial load but not immune activation



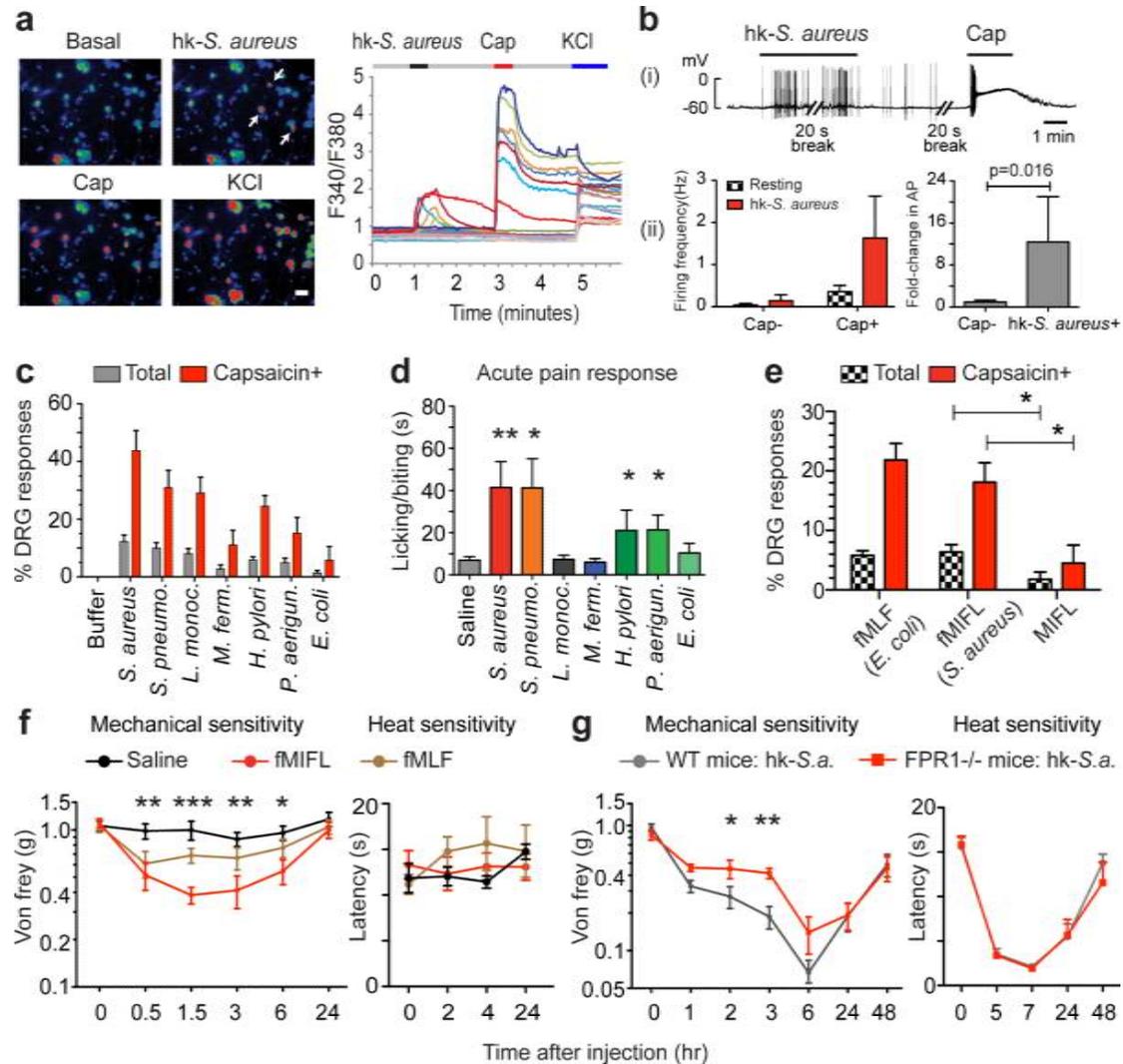
**Innate and adaptive host defenses not  
necessary for *S. aureus* pain**

# Innate immunity through TLR2/MyD88 and neutrophils/monocytes is not necessary for pain during *S. aureus* infection

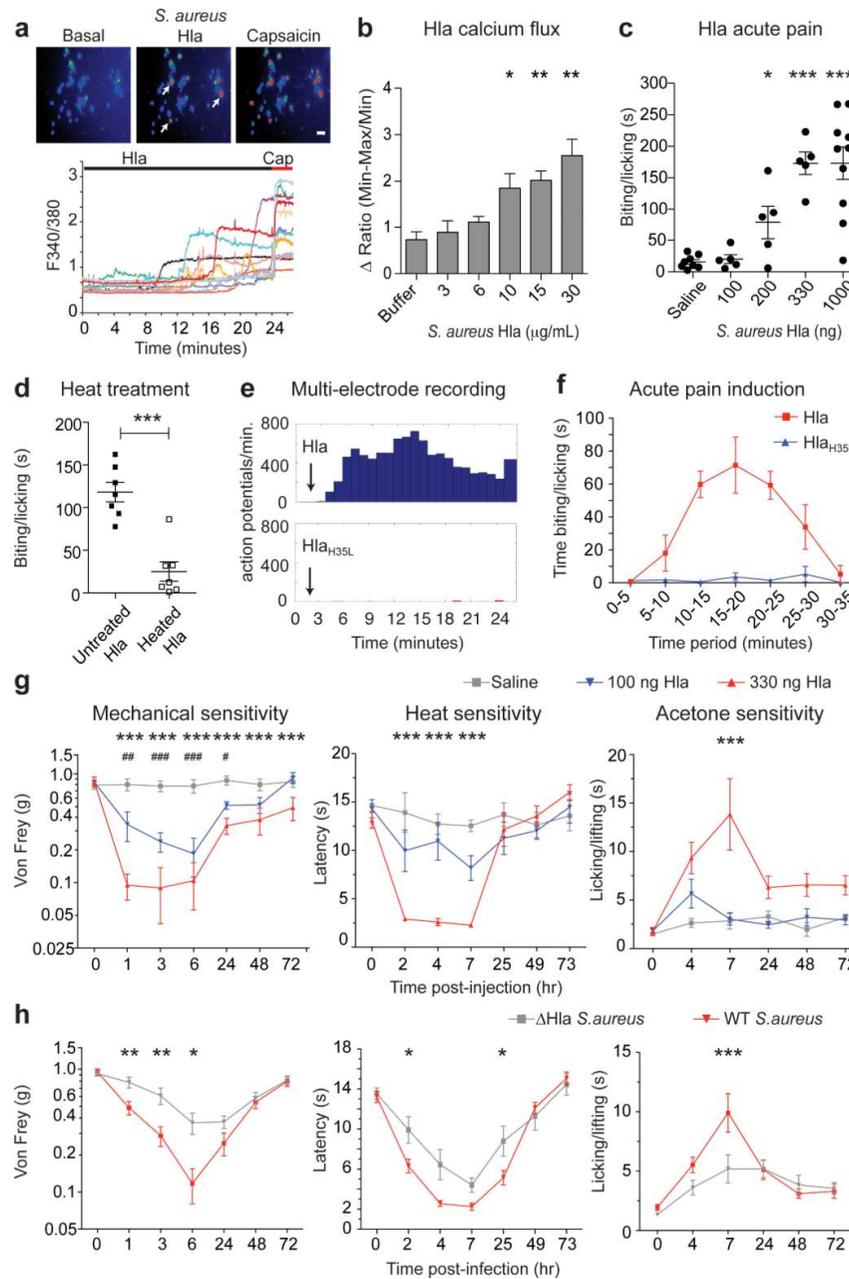


**Formyl peptides and  $\alpha$ -hemolysin  
activate nociceptors**

# Bacterial heat-stable components including N-formylated peptides activate nociceptors

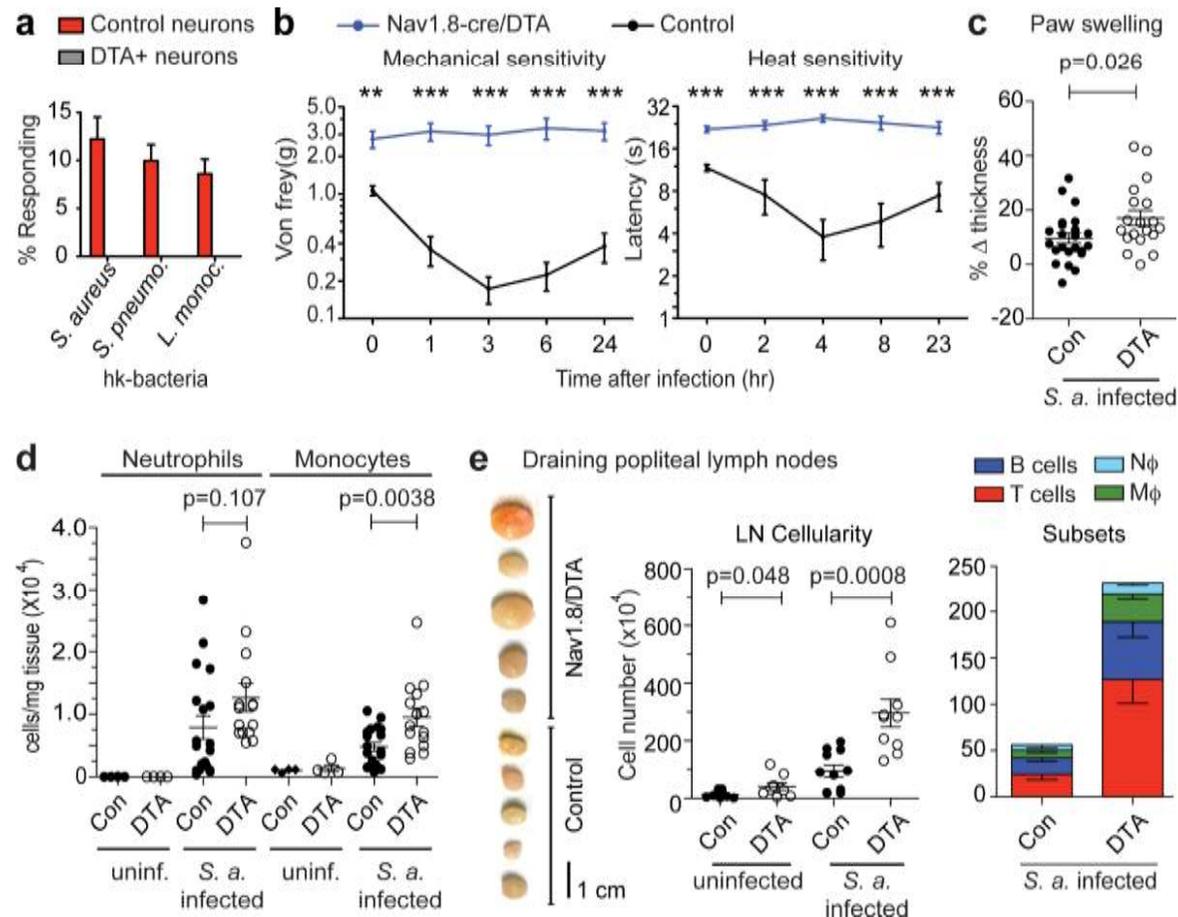


# Heat-sensitive *S. aureus* Hla activates nociceptors and contributes to infection-induced hyperalgesia



# **Nociceptor neuropeptides regulate inflammation**

# Nociceptor ablation leads to increased local inflammation and lymphadenopathy following *S. aureus* infection

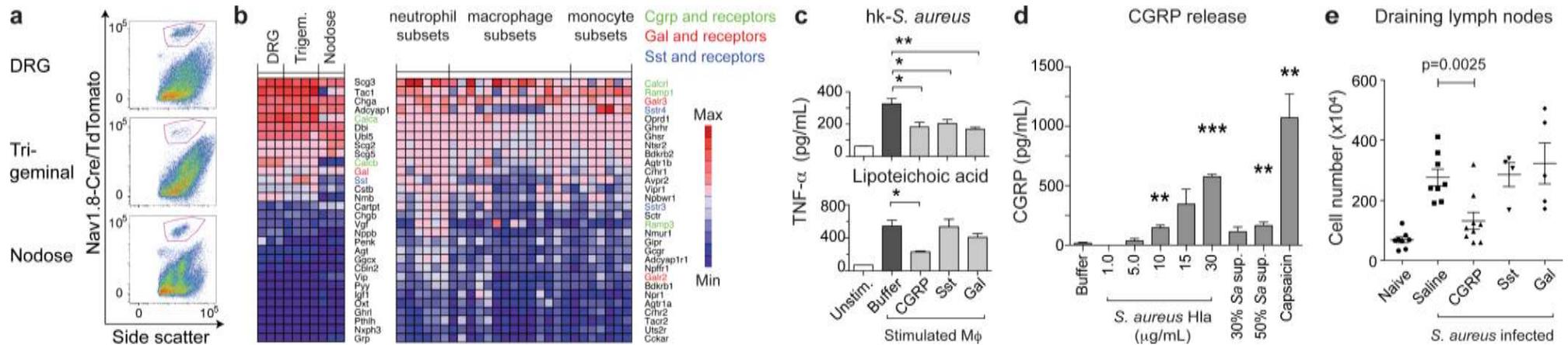


Woolf and collaborators (*Nature 2014*) reported that key immune activation pathways were not necessary for hyperalgesia during acute infection.

Bacteria directly activated nociceptors through N-formyl peptides and the pore-forming toxin alpha-hemolysin (Hla).

Nociceptors release neuropeptides that modulate innate immune activation during infection.

# Nociceptor derived neuropeptides regulate innate immune activation



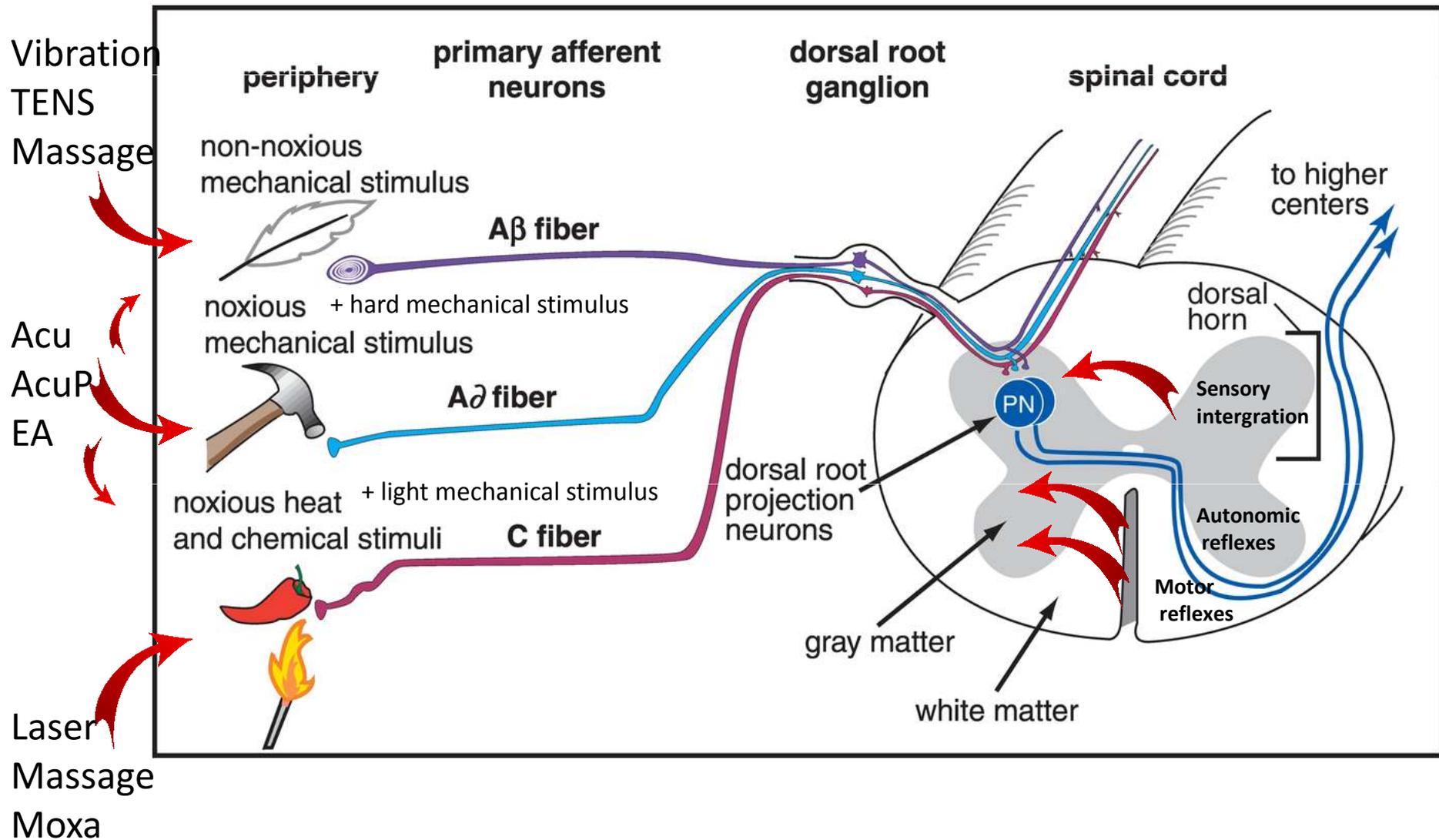
# Peptides released in the periphery during acupuncture

CGRP: Calcitonin Gene-Related Peptide

VIP: Vasoactive Intestinal Polypeptide

Clinical relevance during needling ?

# Peripheral and spinal effects of sensory stimulation



# Peripheral effects of sensory stimulation (EA and TENS) on rat skin

Transcutaneous electrical nerve stimulation (TENS)  
Electro-acupuncture (EA)

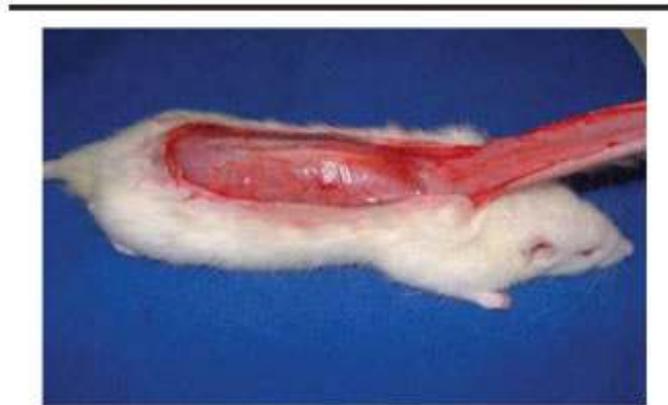
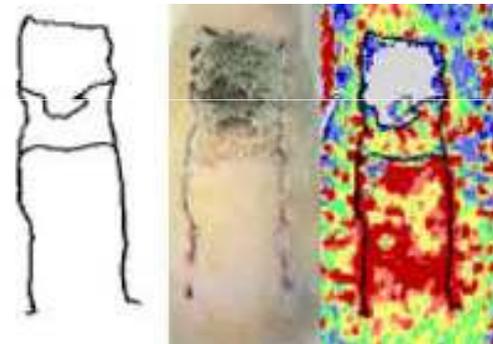
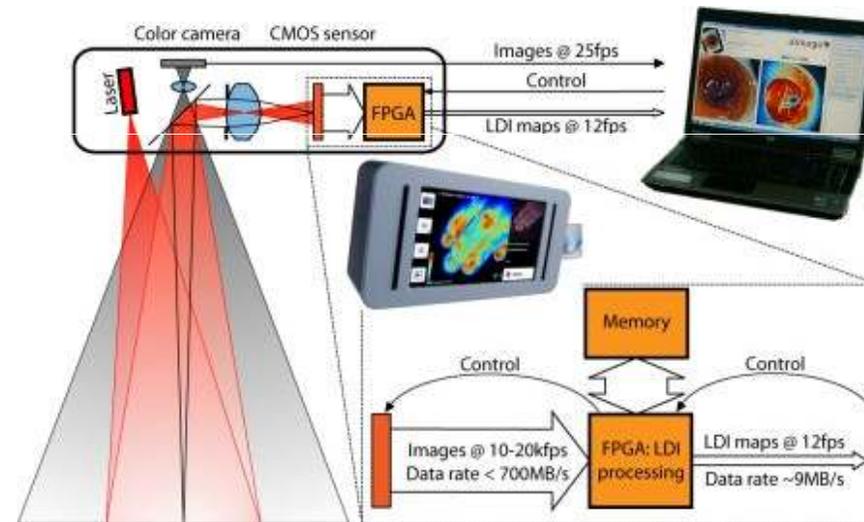


FIGURE 3 – Random skin flap raised.

Random skin flaps



Kjartansson J et al. Calcitonin gene-related peptide (CGRP) and transcutaneous electrical nerve stimulation (TENS) increase cutaneous blood flow in a musculocutaneous flap in the rat. *Acta Physiol Scand.* 1988 Sep;134(1):89-94.

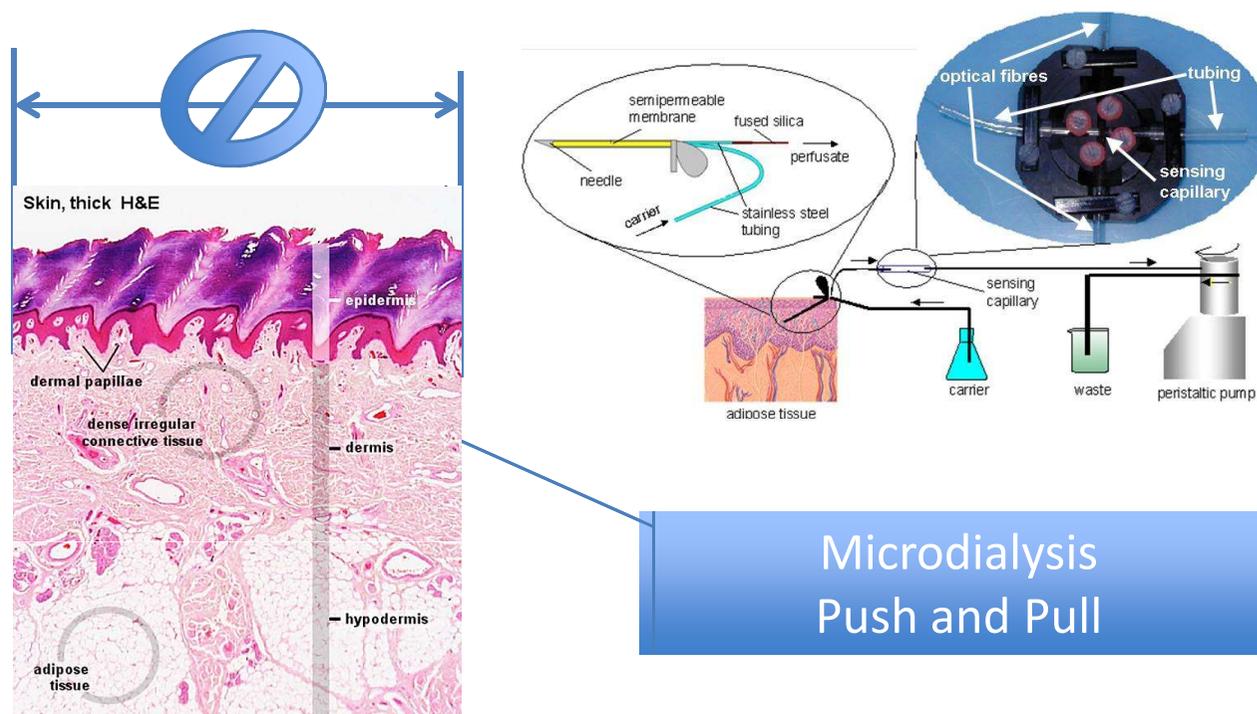
Jansen G et al. Acupuncture and sensory neuropeptides increase cutaneous blood flow in rats. *Neurosci Lett.* 1989 Feb 27;97(3):305-9.

# Effects of electrical nerve stimulation on vasoactive mediators in rat skin



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Electrical stimulation of rat skin

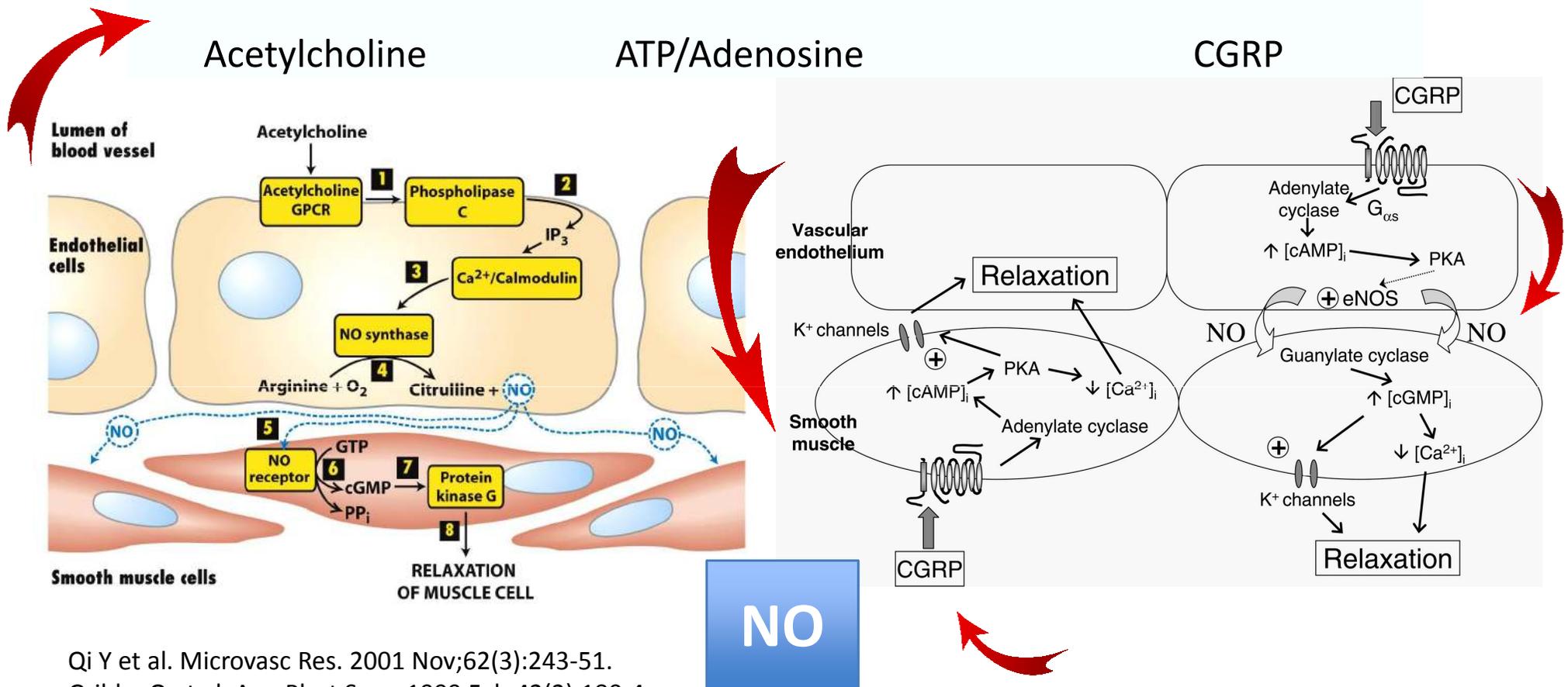


Increased levels of CGRP, VIP, Acetylcholine, ATP, Adenosine, NO

Qi Y et al. Microvasc Res. 2001 Nov;62(3):243-51; Gribbe O et al. Ann Plast Surg. 1999 Feb;42(2):180-4.  
Gribbe O et al. Br J Plast Surg. 1997 Oct;50(7):483-90.

# Electrical stimulation induced smooth muscle relaxation

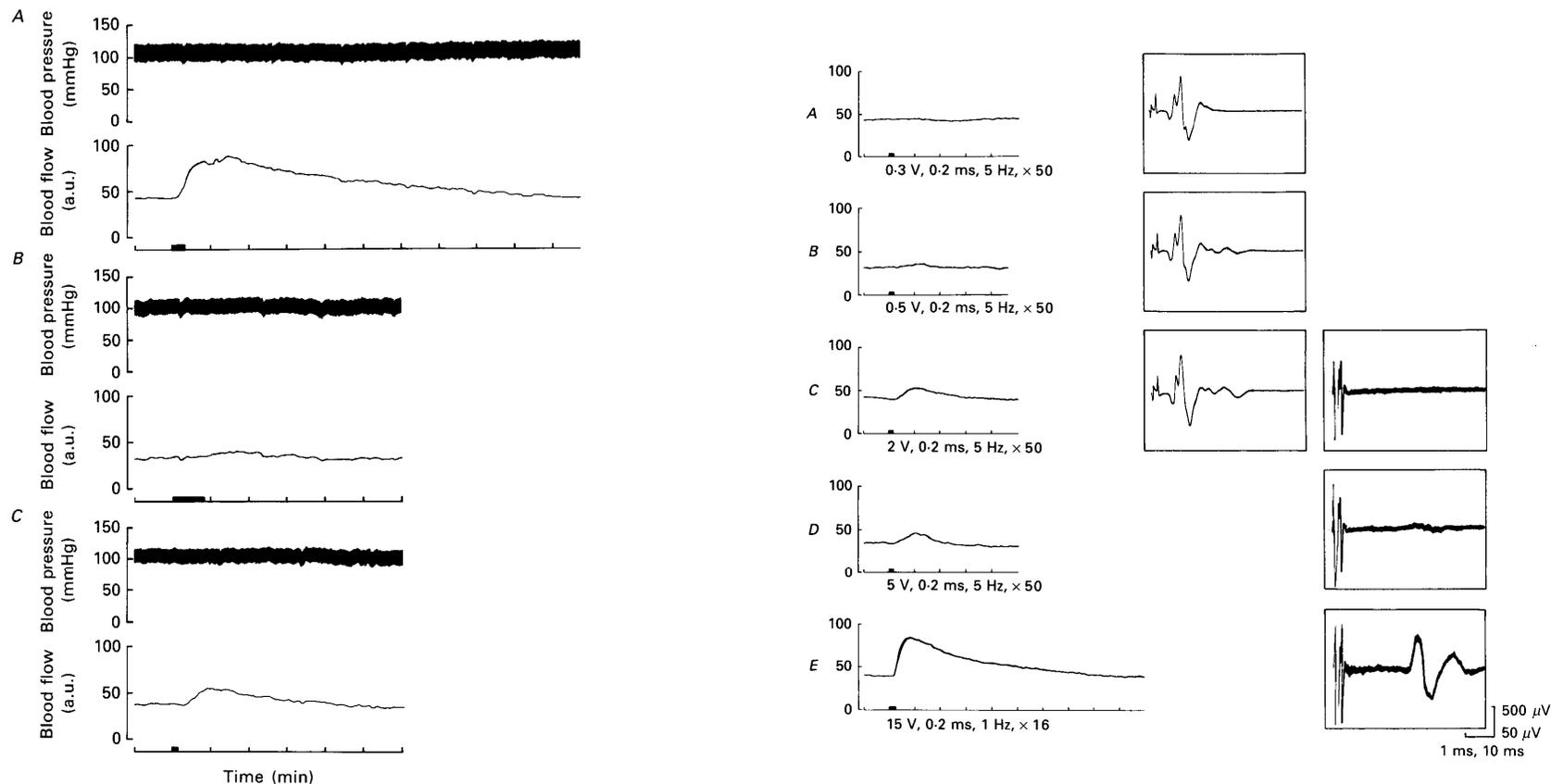
Smooth muscle relaxation – vasodilation – increased nutritive blood flow



Qi Y et al. Microvasc Res. 2001 Nov;62(3):243-51.  
 Gribbe O et al. Ann Plast Surg. 1999 Feb;42(2):180-4.  
 Gribbe O et al. Br J Plast Surg. 1997 Oct;50(7):483-90.

# Peripheral effects of sensory stimulation (EA and TENS) on rat skin

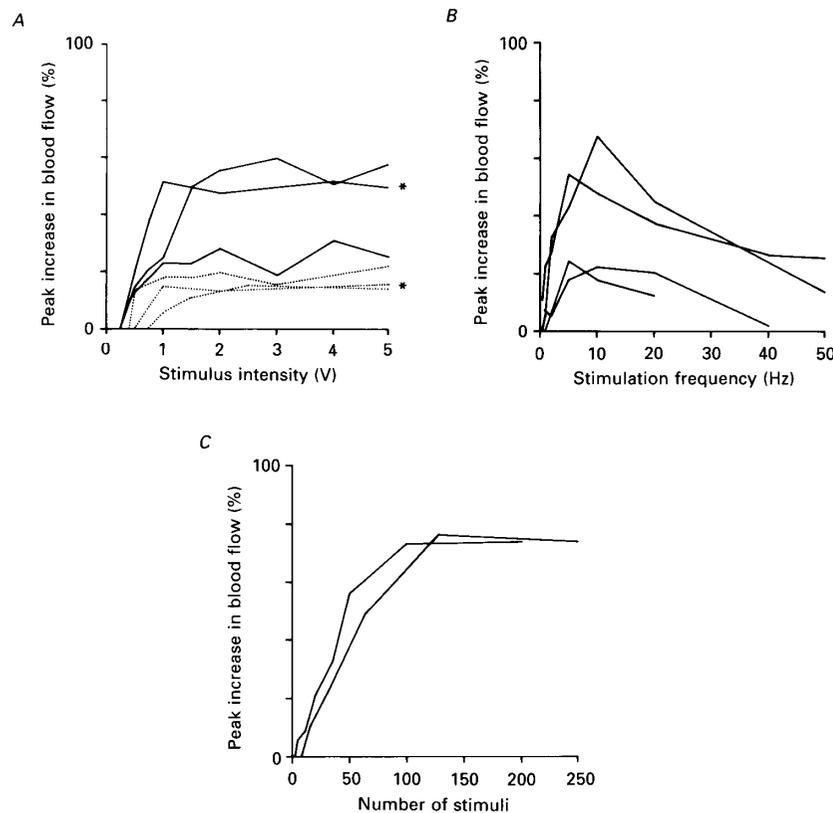
Small diameter myelinated afferents produce vasodilation but not plasma extravasation in rat skin



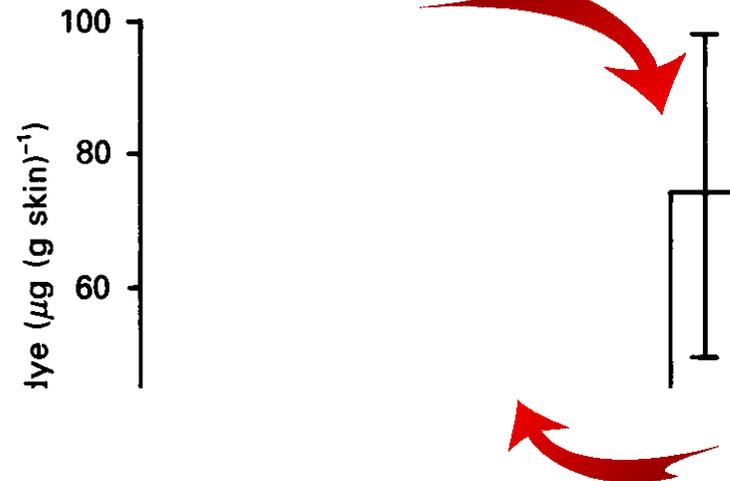
Jänig et al. Small diameter myelinated afferents produce vasodilation but not plasma extravasation in rat skin  
J Physiol 1989, 415:477-486-9.

# Peripheral effects of sensory stimulation (EA and TENS) on rat skin

Small diameter myelinated afferents produce vasodilation but not plasma extravasation in rat skin



the frequency and number of stimuli had any effect turned out to be the case on both counts and the fig. 3B and C. They show that the greatest skin vasodilation with antidromic activation of A $\delta$ -fibres occurs when stimuli are delivered at 5–10 Hz. Under these optimal conditions, responses ranged from 14 to 24 s (mean = 18 s in four animals).



# Conclusion

Acupuncture and Electro-acupuncture induce a local vasodilatory response in the skin.



Can this effect be used in clinical practise in the treatment of acute ischemia/ischemic inflammation in the skin?

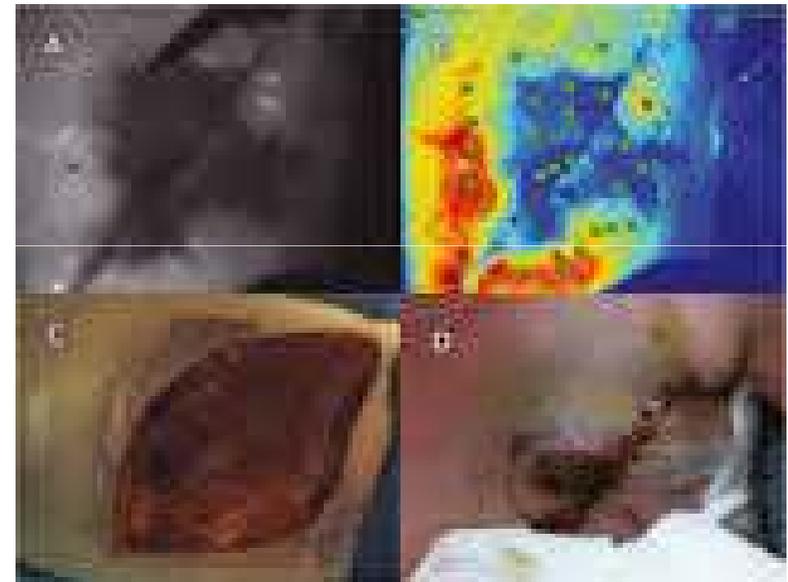
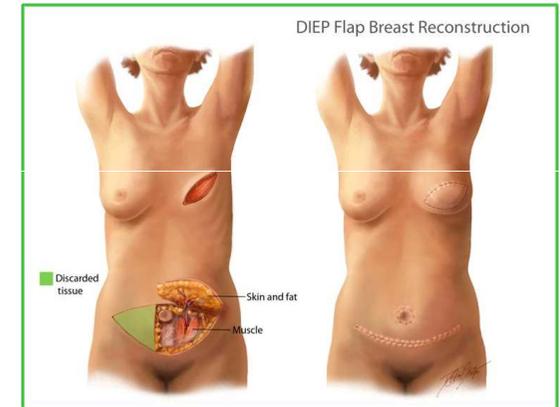


# Clinical effects of sensory stimulation (ENS) on human skin flaps

Blood circulation was measured by laser doppler flowmetry in fasciocutaneous flaps of 24 patients who underwent reconstructive surgery for mammary carcinoma.

19 of the 24 patients had clinical signs of deficient circulation in the flaps. 14 patients were treated with electrical nerve stimulation (ENS) and 10 with placebo-ENS. Varying degrees of necrosis developed in 8 of the 10 patients who received placebo stimulation but in none of those treated with ENS.

In the 5 patients with good capillary refilling and no signs of stasis or oedema before treatment, only minor increases in blood flow occurred after ENS.



# Clinical effects of sensory stimulation (ENS) on human skin flaps



Blood flow was measured in the skin flaps of 20 patients who had undergone reconstructive surgery.

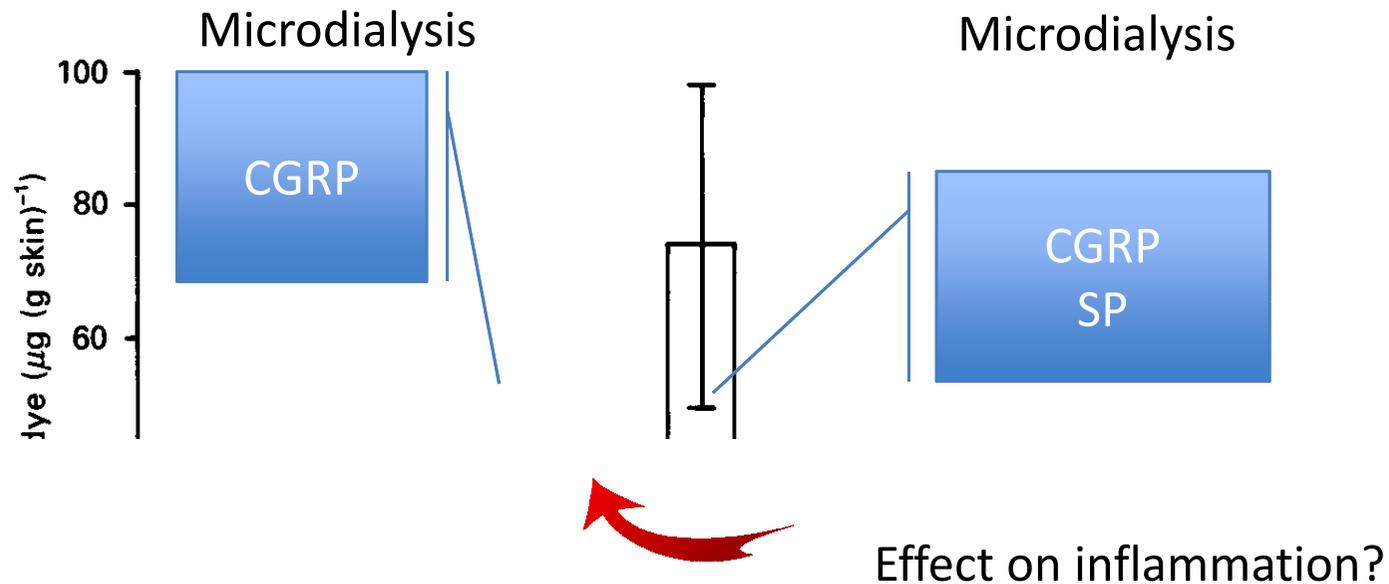
All flaps were showing clinical signs of deficient circulation.

Local blood flow in skin flaps was significantly increased by electrical nerve stimulation (ENS) ( $p$  less than 0.001), but not by placebo ENS.

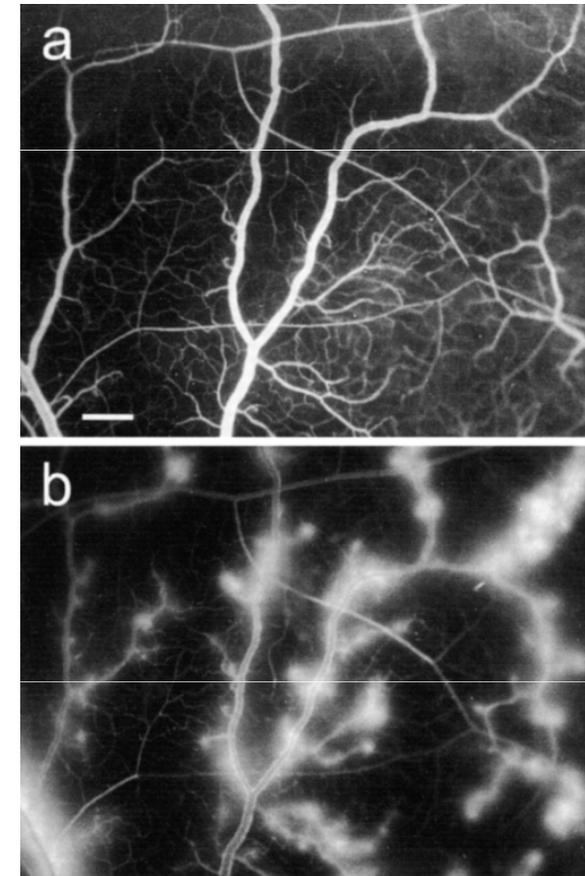
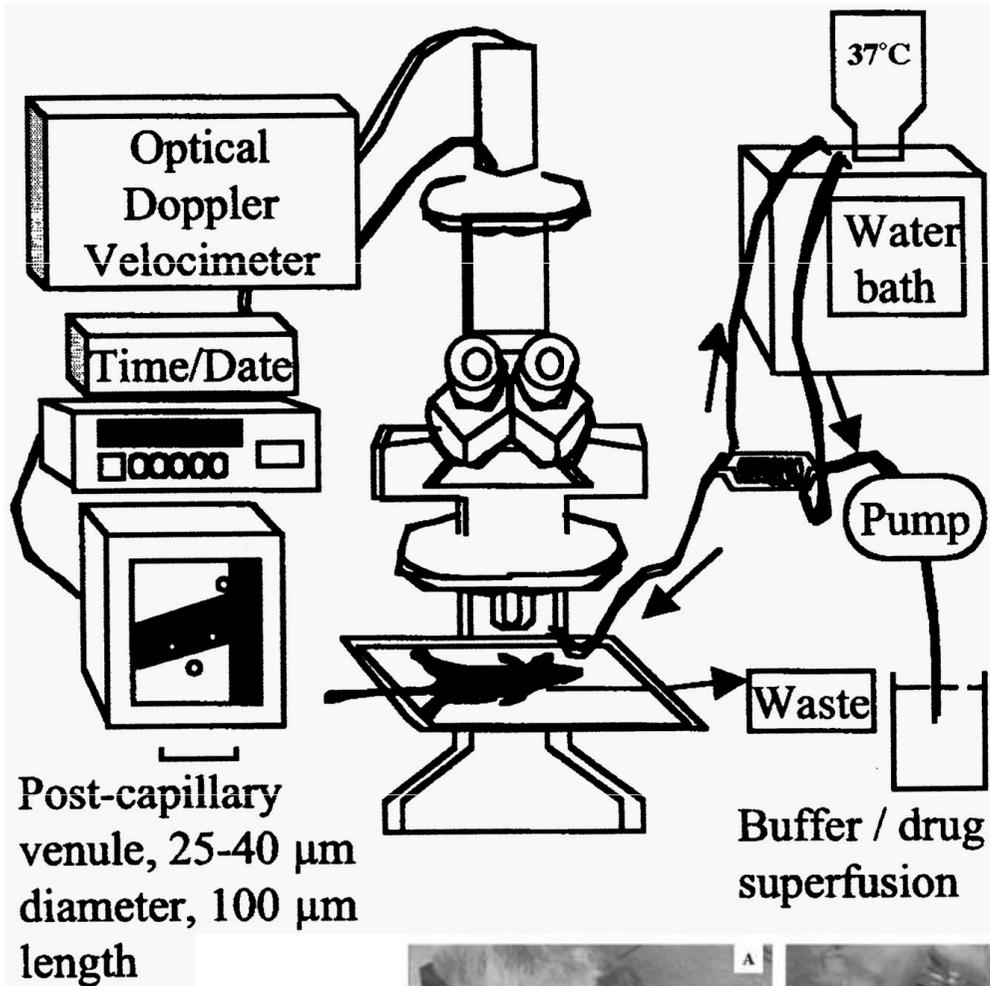
Repeated ENS treatment reduced stasis and oedema significantly ( $p$  less than 0.001), and the capillary refill was also significantly improved ( $p$  less than 0.001).

# Microdialysis

the frequency and number of stimuli had any effect. It turned out to be the case on both counts and the skin temperature (Fig. 3B and C). They show that the greatest skin temperature increases were obtained with antidromic activation of A $\delta$ -fibres once stimuli are delivered at 5–10 Hz. Under these optimal conditions, temperature responses ranged from 14 to 24 s (mean = 18 s from four animals).



# Intravital microscopy



In vivo fluorescence micrographs of the hamster cheek pouch

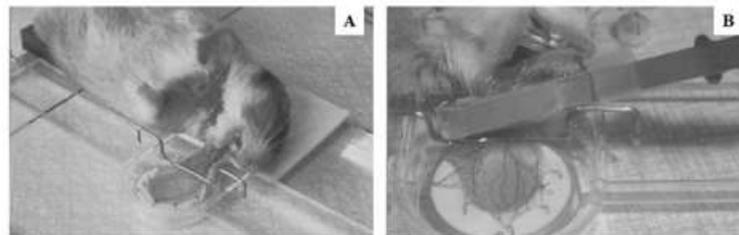


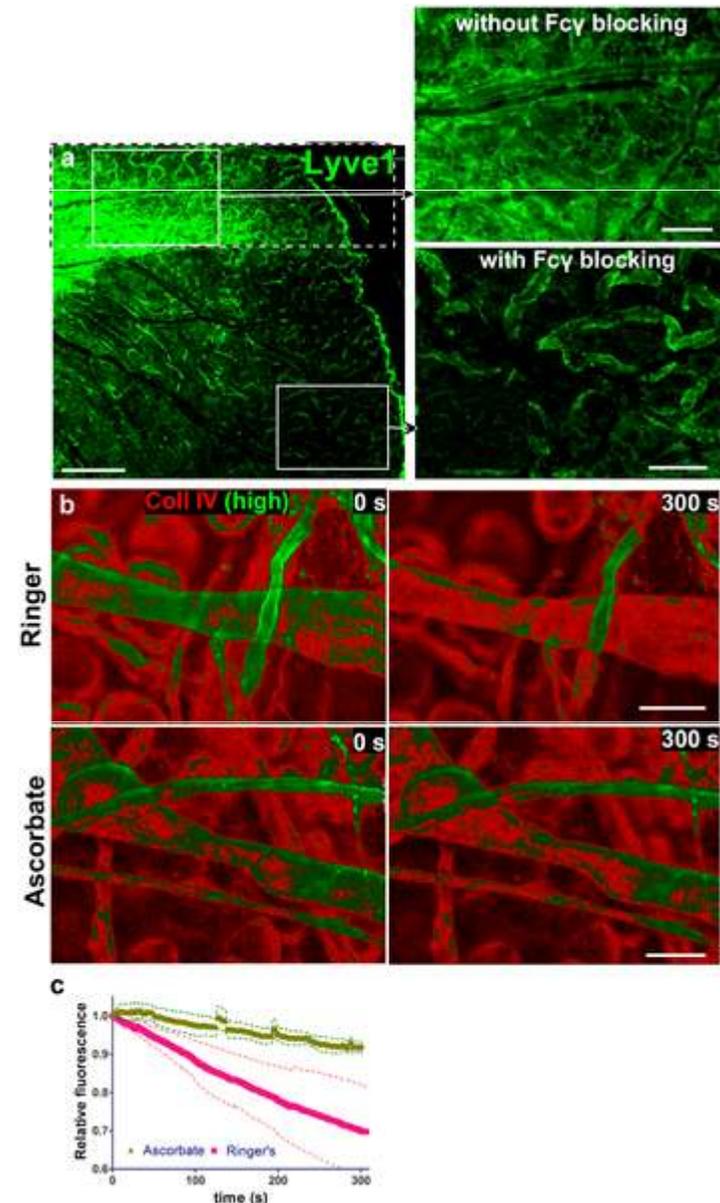
Figure 2 - A. Hamster cheek pouch (HCP) model. B. The inflatable cuff mounted around the neck of the prepared cheek pouch (HCP).

# Intravital Immunofluorescence

Calcitonin gene-related peptide (CGRP), but not substance P (SP), was found to inhibit edema-promoting actions of inflammatory mediators (histamine, leukotrine B4, 5-hydroxytryptamine) in vivo in the hamster cheek pouch, human skin, and rat paw.

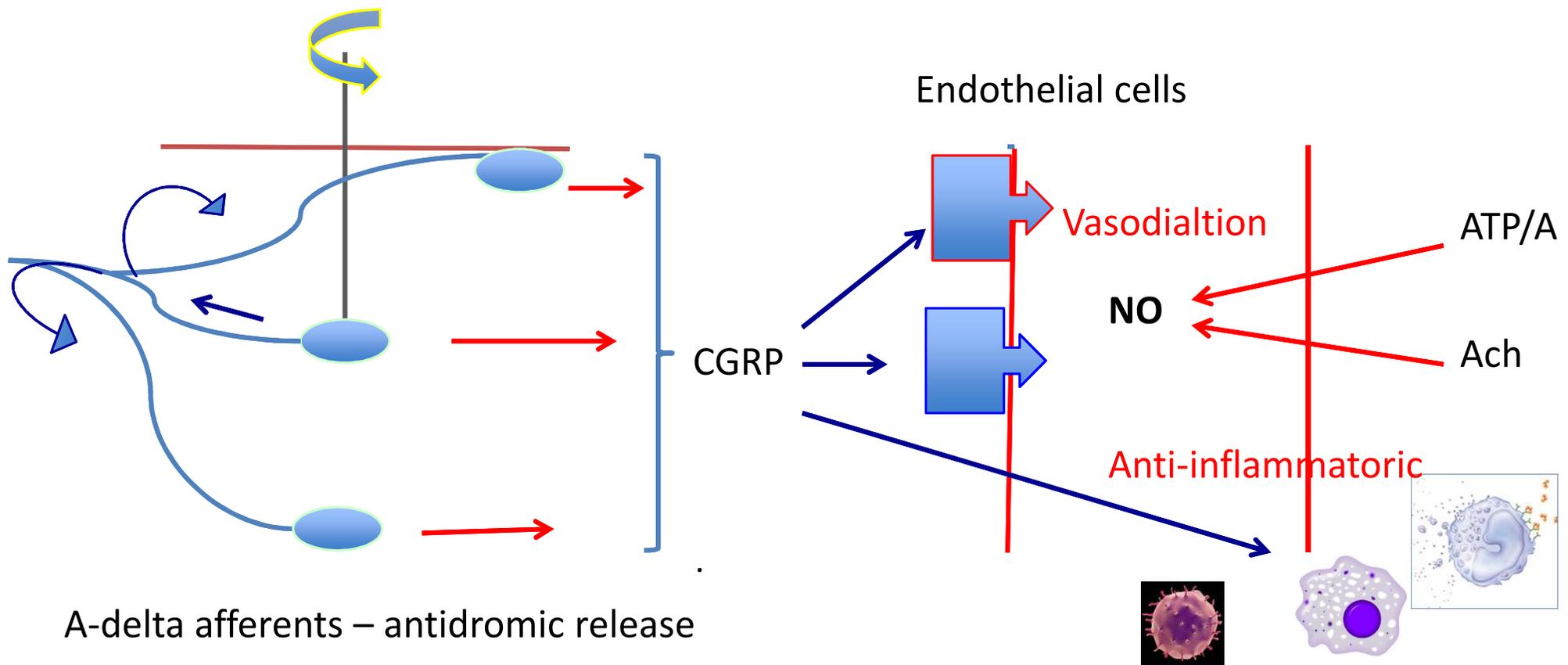
The effect of CGRP was present in the low nanomolar dose range, and it was mimicked by activation of sensory nerves with capsaicin which caused release of endogenous CGRP-like immunoreactivity (IR).

Raud J, Lundeberg T, Brodda-Jansen G, Theodorsson E, Hedqvist P. Potent anti-inflammatory action of calcitonin gene-related peptide. *Biochem Biophys Res Commun.* 1991 Nov 14;180(3):1429-35.



# Suggested mechanisms of action

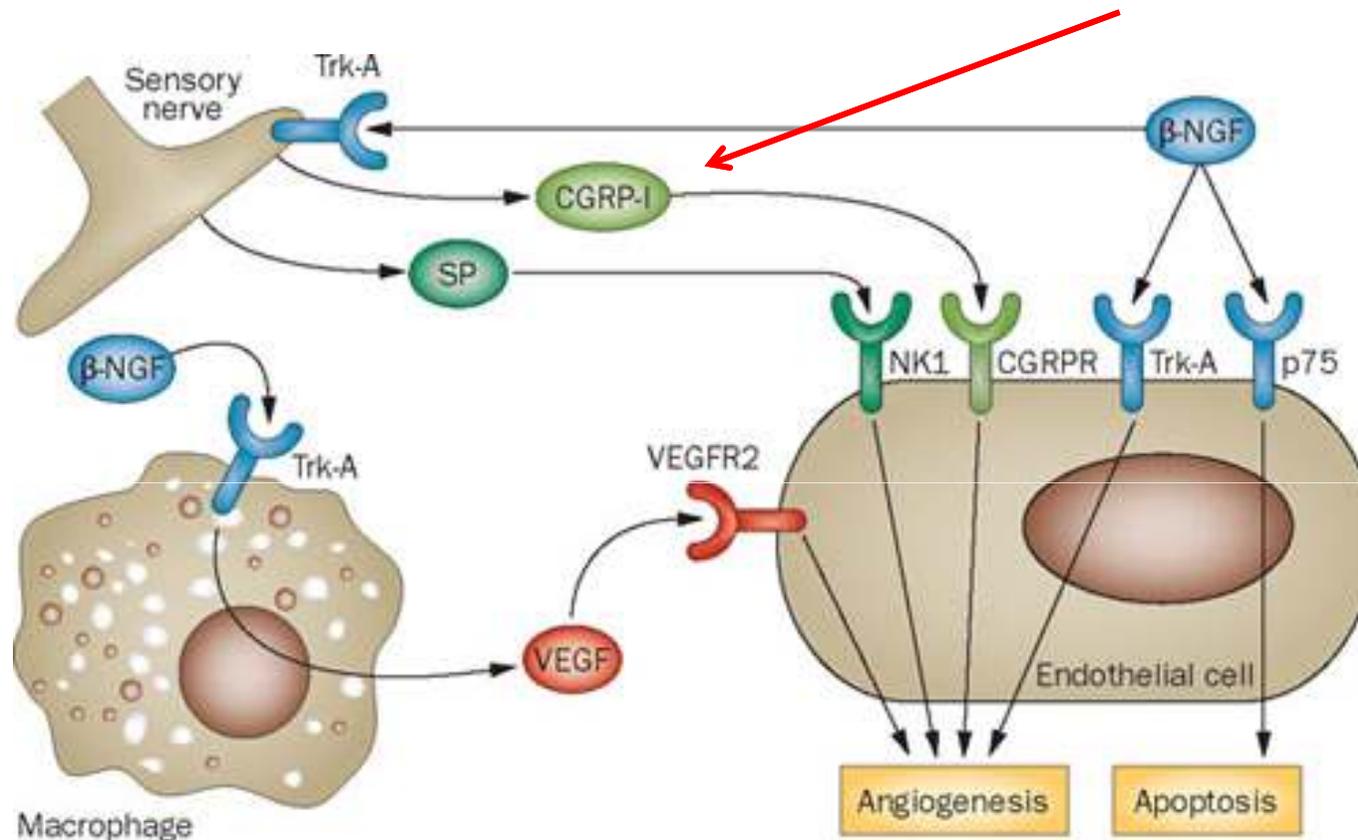
Acupuncture and Electro-Acupuncture activates afferent A-delta fibers in the skin  
Thereby inducing the release of CGRP that results in vasodilation and an anti-inflammatory effect



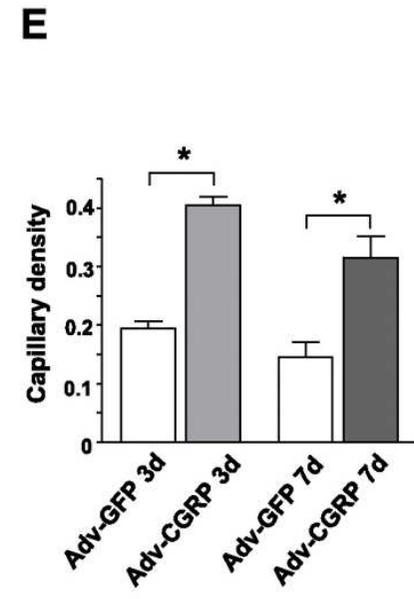
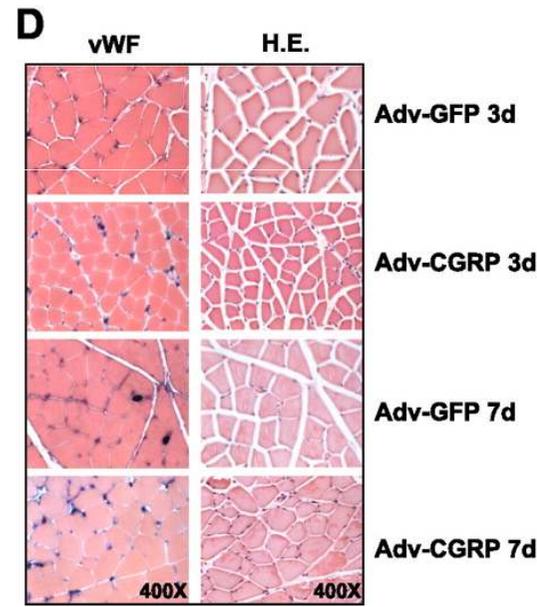
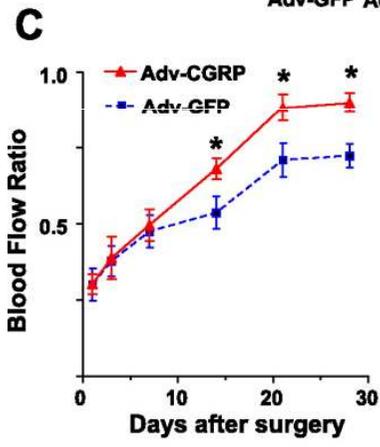
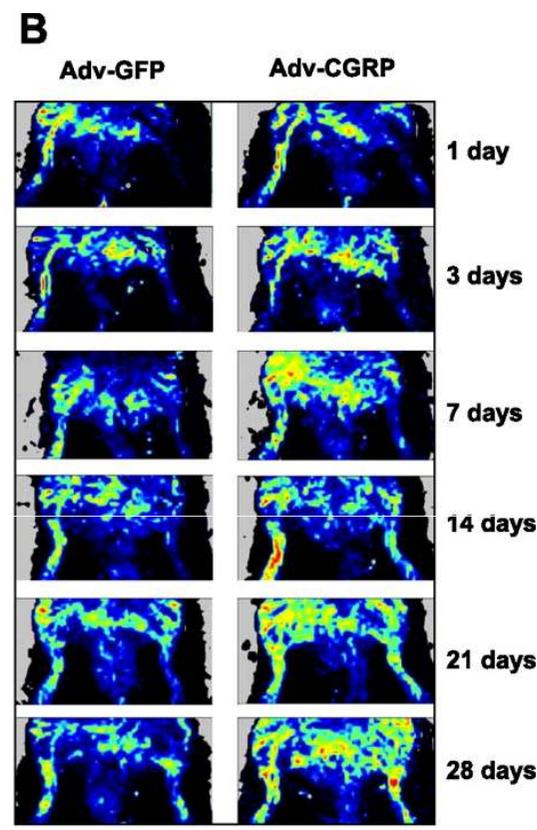
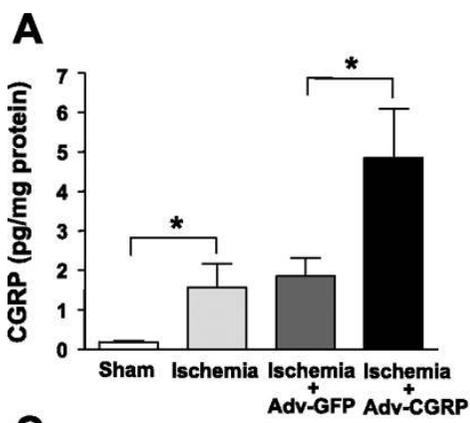
A-delta afferents – antidromic release

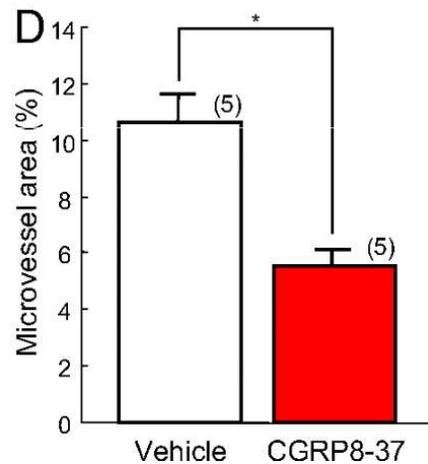
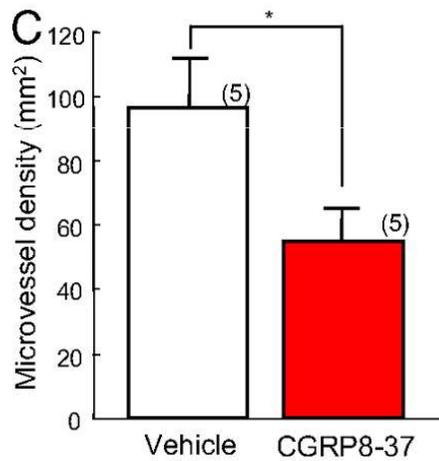
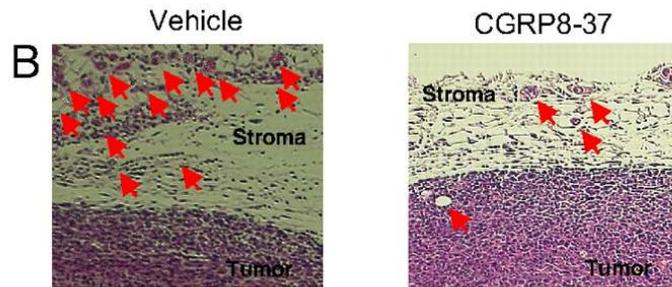
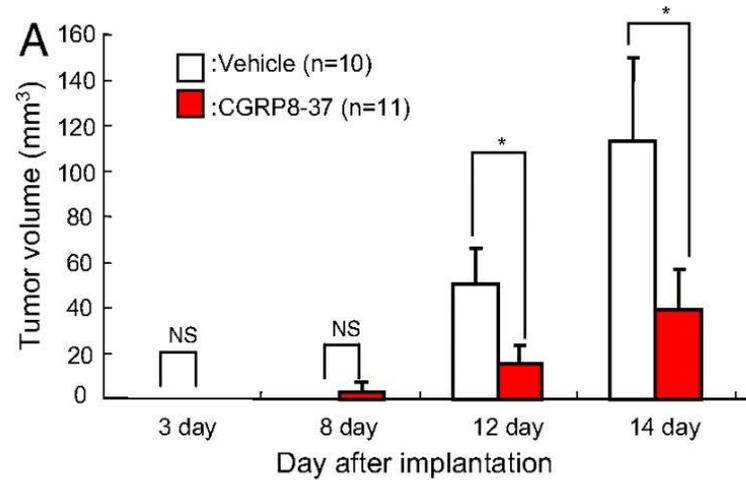
# Suggested mechanisms of action

Acupuncture and Electro-Acupuncture activates afferent A-delta fibers in the skin thereby inducing the release of CGRP that results in angiogenesis – healing.



# CGRP promotes angiogenesis via AMP-activated protein kinase





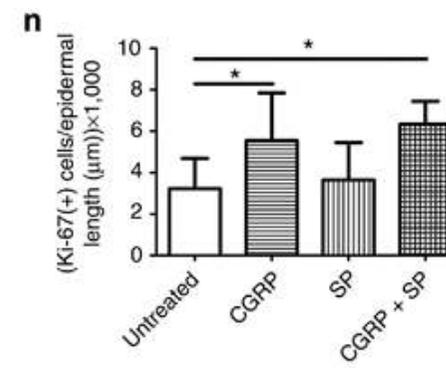
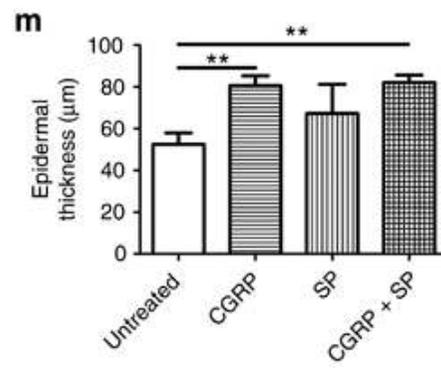
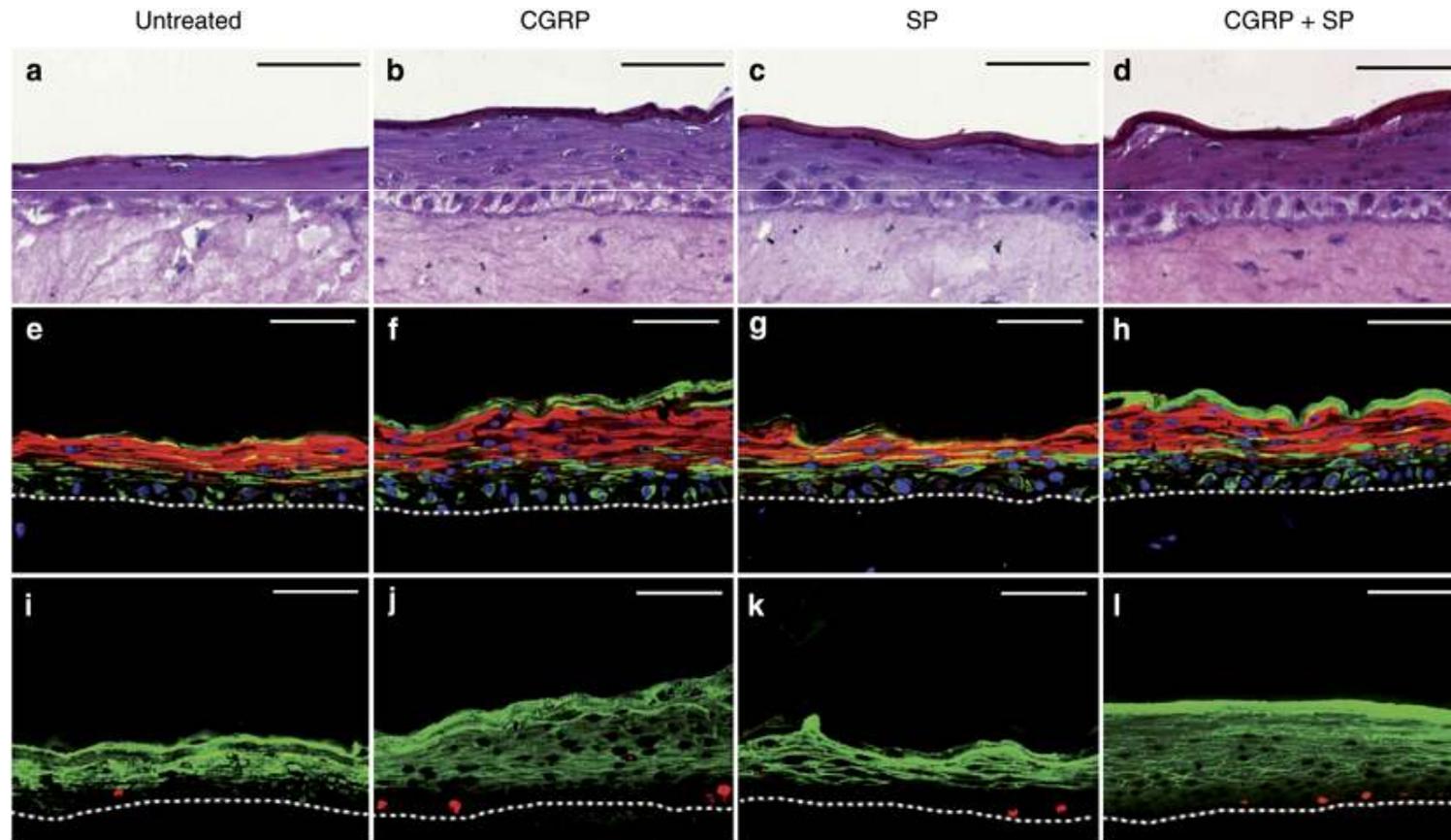
# CGRP promotes angiogenesis

Neuronal system-dependent facilitation of tumor angiogenesis and tumor growth by calcitonin gene-related peptide

Masaya Toda et al. PNAS 2008 vol. 105 no. 36, 13550–13555

# Neuron-induced thickening of the epidermis depends on calcitonin gene-related peptide (CGRP)

*Journal of Investigative Dermatology* (2013) **133**, 1620–1628

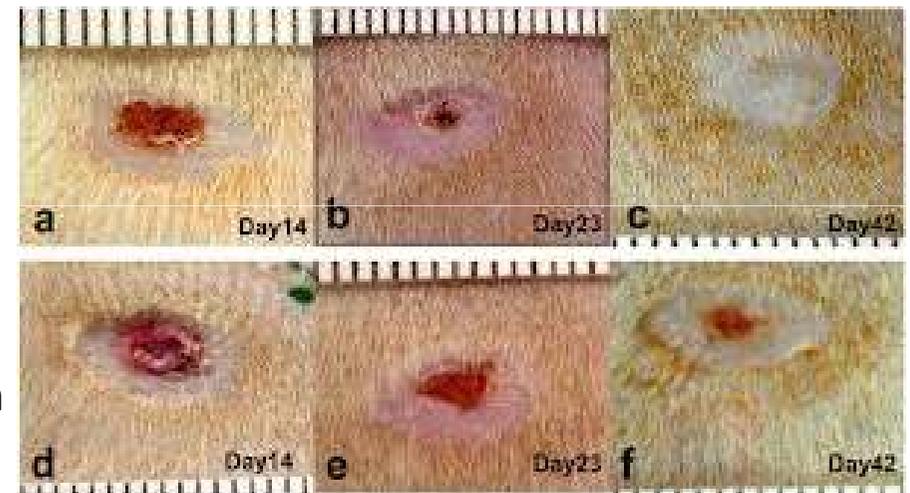
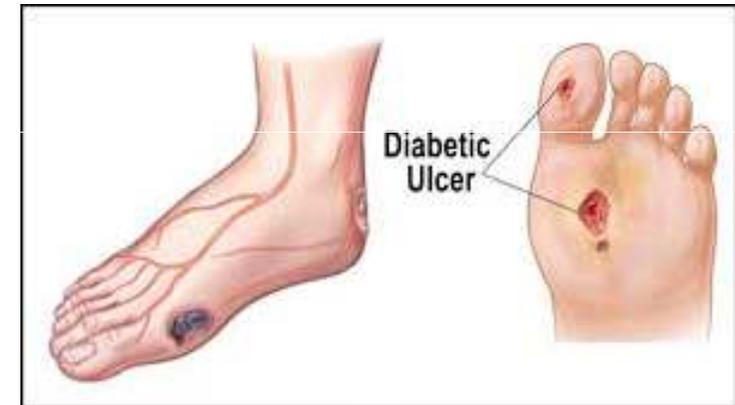


# Clinical effects of electrical nerve stimulation

A controlled study of the effects of electrical nerve stimulation (ENS) was performed in conjunction with a standard treatment for healing chronic diabetic ulcers on **64 patients** divided randomly into two groups.

All patients received **standard treatment** (paste-impregnated bandage and a self-adhesive elastic bandage) **plus placebo ENS** or **ENS** (alternating constant current; frequency, 80 Hz; pulse width, 1 msec; intensity-evoking strong paresthesias) for 20 minutes twice daily for 12 weeks.

Comparison of percentages of healed ulcer area and the number of healed ulcers was made after 2, 4, 6, 8, and 12 weeks. There were significant differences ( $p < 0.05$ ) in both ulcer area and healed ulcers in the ENS group compared with the placebo group after 12 weeks of treatment.



## Clinical effects of CGRP + VIP on human ulcers

A study on the effects of iontophoretic administration of calcitonin gene-related peptide and vasoactive intestinal polypeptide on the healing of venous stasis ulcers of the extremities was carried out on 66 patients.

Two randomized groups of patients were compared, one receiving **standard treatment plus iontophoresis of calcitonin gene-related peptide and vasoactive intestinal polypeptide**, and the other receiving standard treatment plus **placebo iontophoresis**. Calcitonin gene-related peptide and vasoactive intestinal polypeptide were administered locally by iontophoresis for 20 min three times weekly for 12 weeks.

To determine the effects of the two treatments, the percentage surface area of ulcer healed and the number of healed ulcers were compared after 2, 4, 6, 8, and 12 weeks of treatment.

The results demonstrate that there were significant improvements of the healing process in the group treated with calcitonin gene-related peptide and vasoactive intestinal polypeptide when compared with placebo iontophoresis.



Venous ulcer

Gherardini G, et al. *Plast Reconstr Surg.* 1998 Jan;101(1):90-3.

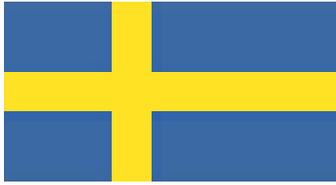


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# Electro-Acupuncture, Exercise and Myokines



# Swedish father of acupuncture



*Early in his career (70s) he became convinced that acupuncture is an effective method of pain control based on the activation of the body's own systems and that needle stimulation (acupuncture) could represent the artificial activation of such systems.*



Sven Andersson (1927-2007).

# Acupuncture: From empiricism to science

*Professor Andersson emphasised the **empirical basis of** Traditional Chinese Medicine and when trying to explain **TCM** he would tell the story of how the Vikings tried to explain lightning and thunder.*

*According to the Vikings, **Thor the god of thunder**, son of Odin and a member of the Aesir, smashed giants' heads with his mighty hammer, thereby causing lightning and thunder: in other words, the lightning and thunder is for real but the rationale given is not.*



# Acupuncture: From empiricism to science

Professor Andersson was convinced that acupuncture could be *integrated into mainstream medicine*, and that a prerequisite for this was that the mechanisms of acupuncture could be *explained in terms of endogenous systems*.

He tried to elucidate *what kind of sensory stimulus was most similar to acupuncture*, and he suggested that acupuncture excites receptors or nerve fibres in the stimulated tissue, which are also physiologically activated by strong muscle contractions, and the effects on certain organ functions are similar to those obtained by *protracted exercise*.

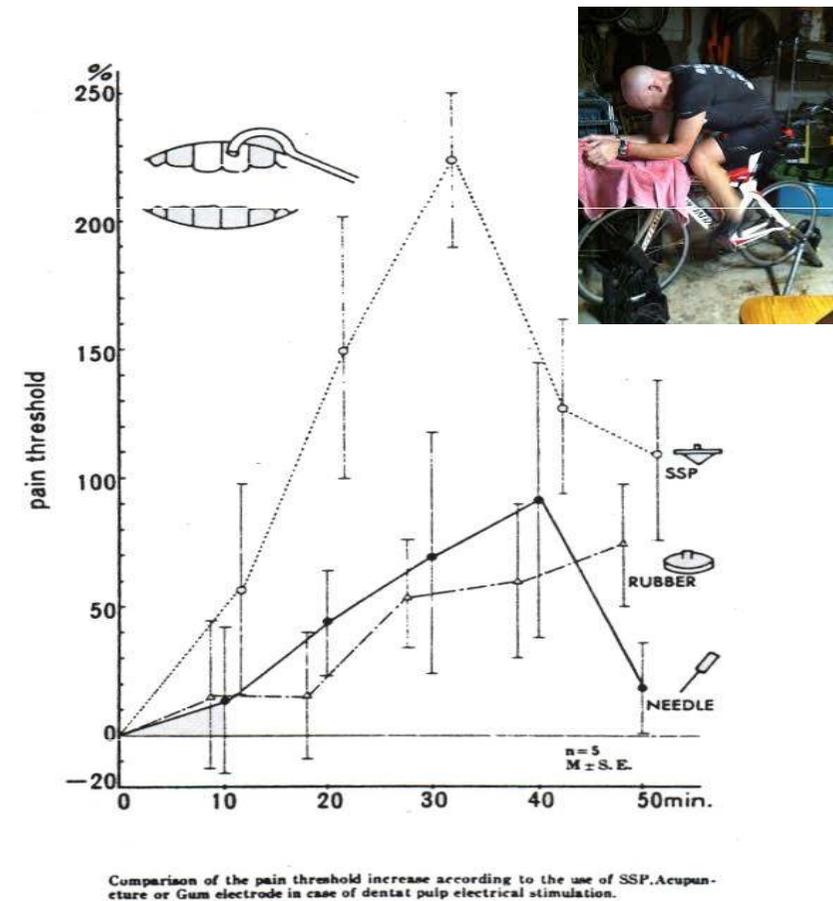


Fig. 3

M Hyodo, T Kitade Surface Acupuncture Point Electrical Treatment With SSP Electrodes, in; *Recent Advances on Acupuncture Treatment*, Pain Clinic, Department of Anaesthesiology, Osaka Medical College, Takatsuki City, Osaka, Japan, 1980

# Acupuncture: From empiricism to science

Professor Andersson also reported that **beta-endorphin levels**, important in pain control as well as in the regulation of blood pressure and body temperature, have been observed to rise in the brain tissue of animals after both acupuncture and strong exercise.

He was also inspired by the fact that experimental and clinical evidence suggest that acupuncture may affect the sympathetic system via mechanisms at **the hypothalamic and brainstem levels**, and that the hypothalamic beta-endorphinergic system has inhibitory effects on the **vasomotor centres**.

He also demonstrated that there was a **post-stimulatory sympathetic inhibition** that reached a maximum effect a few hours after acupuncture and which could be sustained for more than 12 hours. This powerful inhibition of sympathetic tone is probably one of the most important effects of acupuncture in the treatment of diseases.

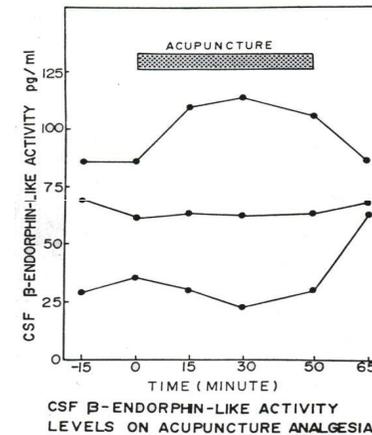
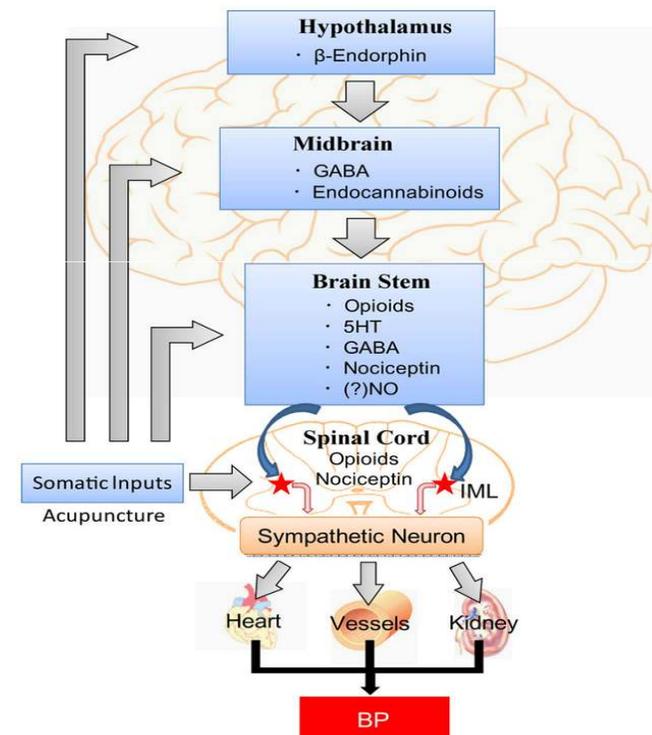
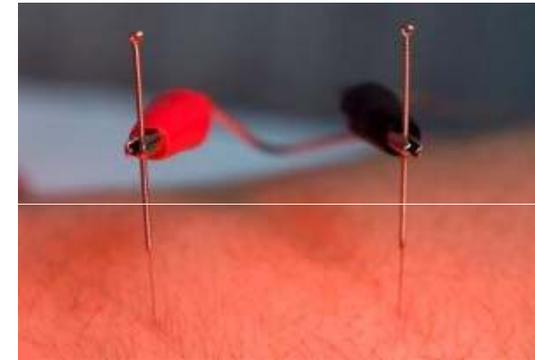


Fig. 6

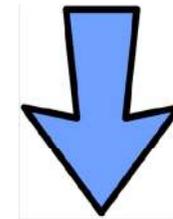
M Hyodo, T Kitade Surface Acupuncture Point Electrical Treatment With SSP Electrodes, in; *Recent Advances on Acupuncture Treatment*, Pain Clinic, Department of Anaesthesiology, Osaka Medical College, Takatsuki City, Osaka, Japan, 1980



# Acupuncture: From empiricism to science

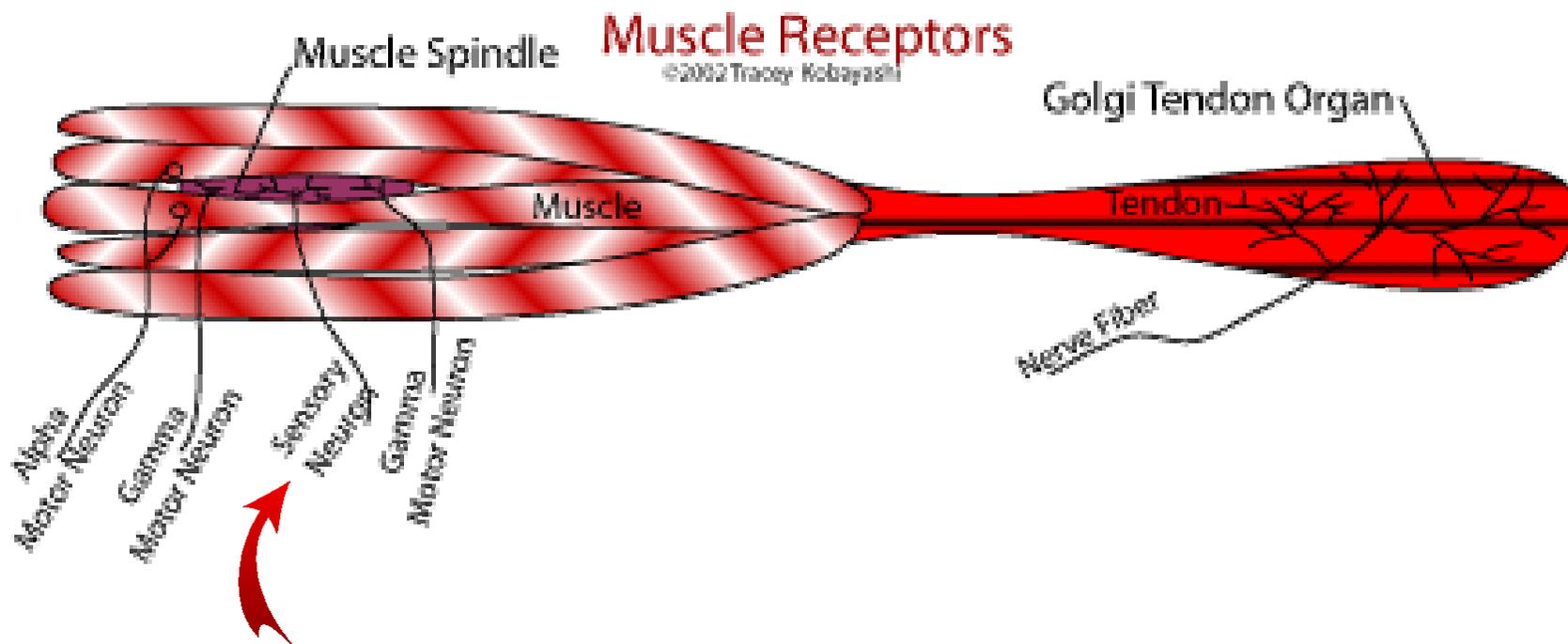


EA



Empowerment

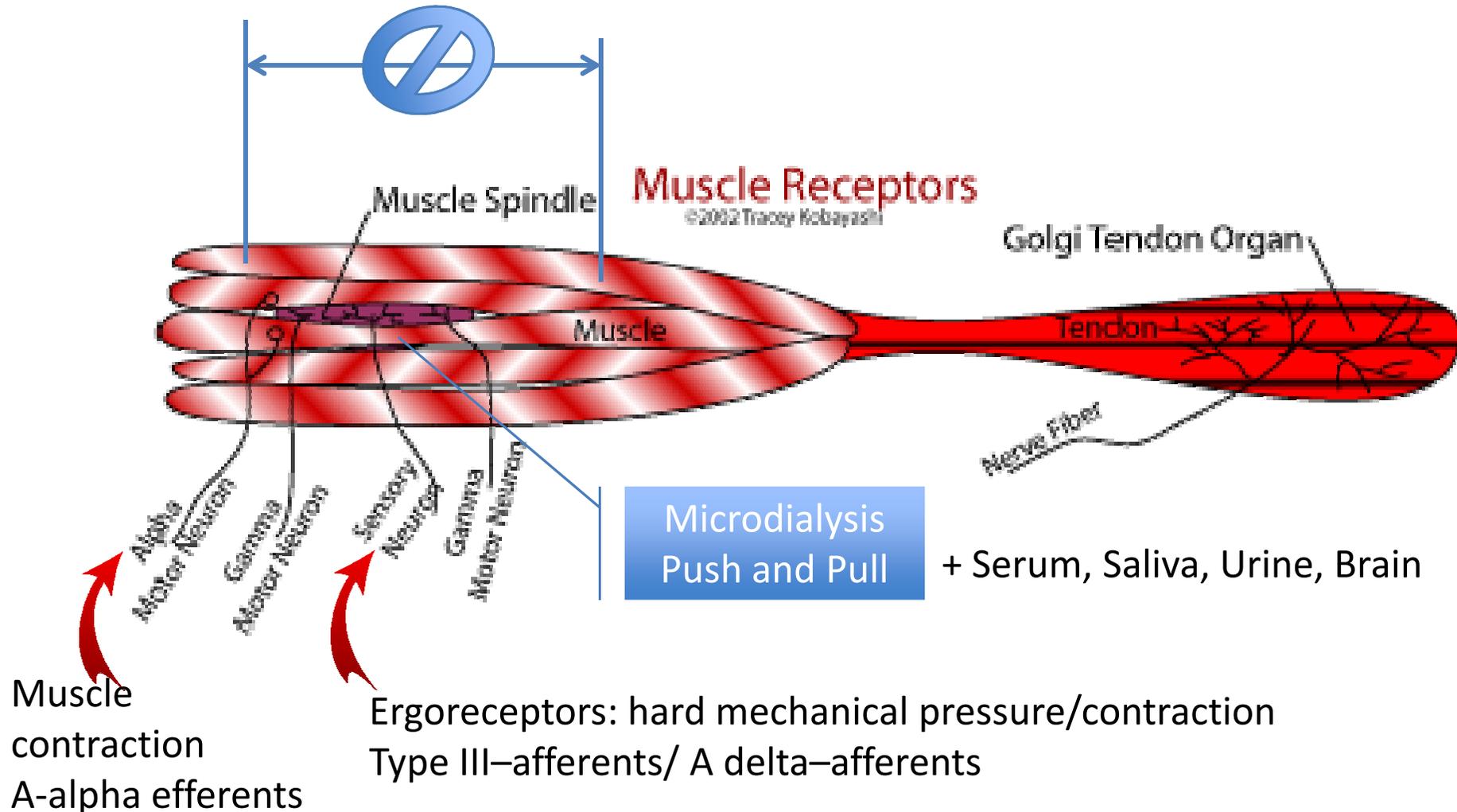
# Peripheral and spinal effects of exercise/acupuncture stimulation



Ergoreceptors: hard mechanical pressure/contraction

# Effects of exercise/acupuncture/ electro-acupuncture stimulation

Manual vs 2 Hz burst EA



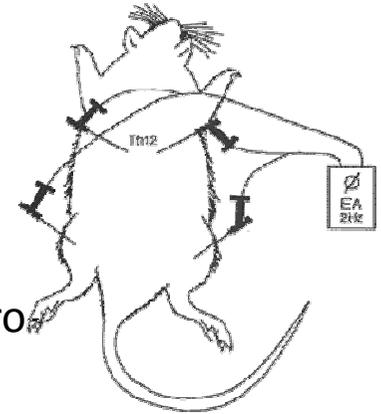
# Effects of exercise/acupuncture/ electro-acupuncture stimulation in rat and human muscles

Increased levels of Calcitonin Gene Related Peptide-like immunoreactivity (CGRP-LI) following exercise (++) and electro-acupuncture (++) but not manual acupuncture. No significant increase in Substance-(SP-LI) or Neurokinin A-like immunoreactivity (NKA-LI) following either modality.

Increased levels of ATP-Adenosine following manual acupuncture (+), electro-acupuncture (+++) and exercise (++++)

Increased levels of Interleukin-6, interleukin-10 following exercise (++) and electro-acupuncture (++) but not manual acupuncture.

Increased levels of Acetylcholine following manual acupuncture (+), electro-acupuncture (+++) and exercise (++++)



Neuropeptides. 1999 Jun;33(3):244-50.

Life Sci. 1998;63(8):659-74.

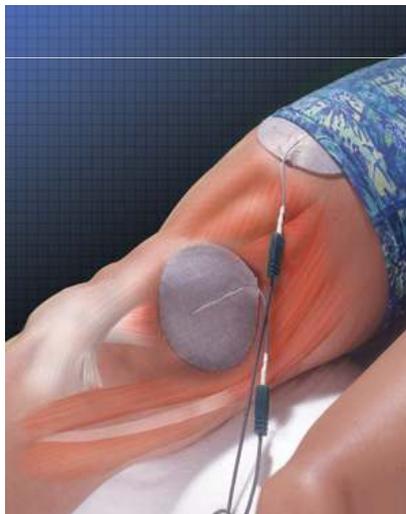
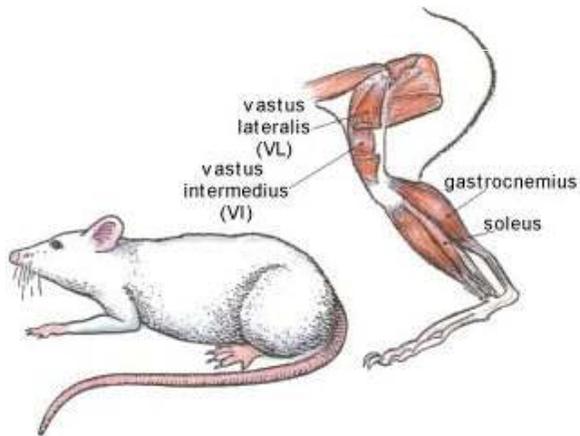
Acta Physiol Scand. 1998 Apr;162(4):517-22.

Unpublished observations



Fig. 3 Wheel running distance of SPORTS rats

# Effects of exercise/acupuncture/ electro- acupuncture stimulation in rat and human muscles



# Myokines



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The term **myokine** refers to cytokines and other peptides that are produced, expressed, and released by muscle fibers and exert either autocrine, paracrine or endocrine effects.

Of particular interest is the fact that contractile activity plays a role in regulating the expression of these cytokines in skeletal fibers.

**Myostatin** was the first myokine to be identified in 1997.

Both aerobic exercise and strength training in humans and animals attenuate myostatin expression and myostatin inactivation.

Myostatin inactivation potentiates the beneficial effects of endurance exercise on metabolism

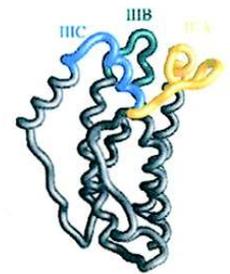
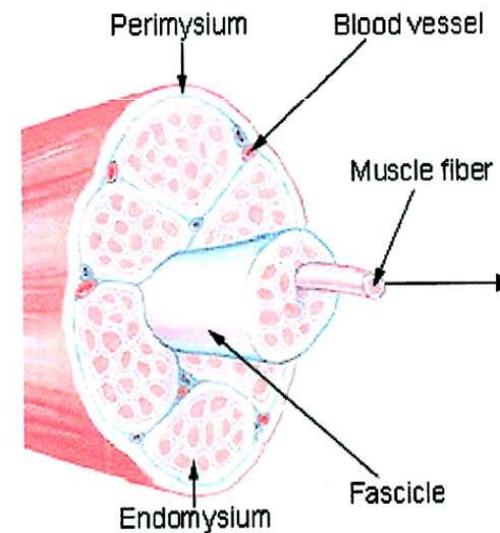
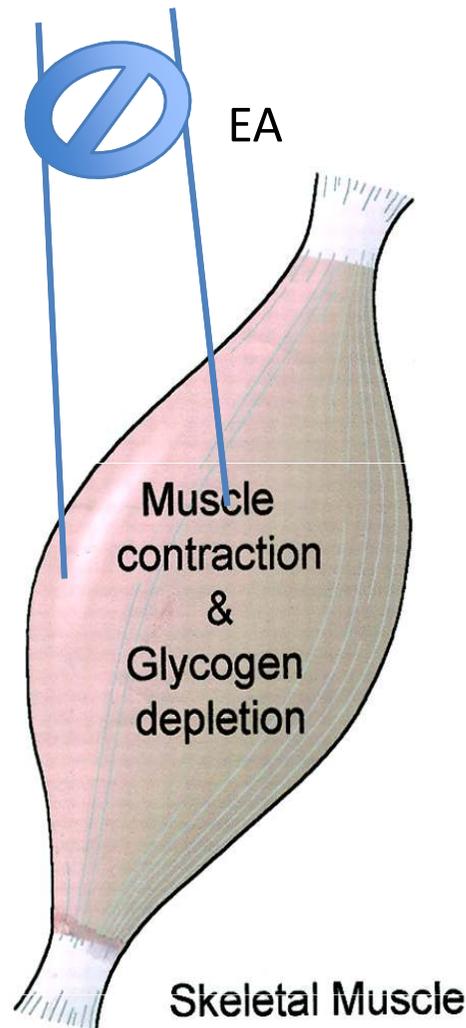
DL, Cleary AS, Speaker KJ, Lindsay SF, Uyenishi J, Reed JM, Madden MC, Mehan RS. "Myostatin, activin receptor IIb, and follistatinlike-3 gene expression are altered in adipose tissue and skeletal muscle of obese mice." *Am J Physiol Endocrinol Metab* 294: E918-E927, 2008.

Bente Klarlund Pedersen, Thorbjörn C. A. Åkerström, Anders R. Nielsen, Christian P. Fischer. "Role of myokines in exercise and metabolism." *Journal of Applied Physiology* | Published 1 September 2007 Vol. 103no. 1093

## Interleukin-6

Interleukin-6 (IL-6) (a gp 130 receptor cytokine) was the first myokine that was found to be **secreted** into the **blood stream** in response to **muscle contraction**

And following 2 Hz burst **Electro-acupuncture** but not Acupuncture

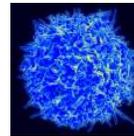


IL-6

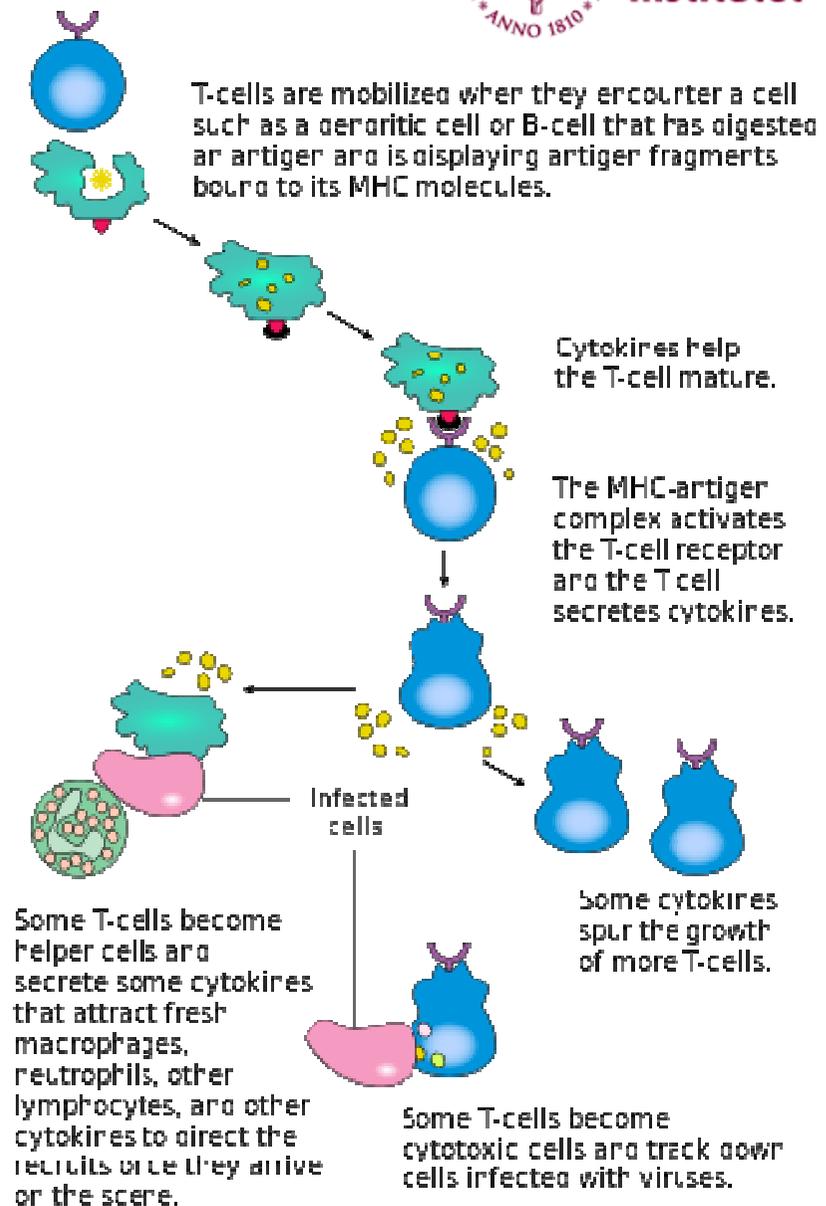
Pedersen BK, Febbraio MA. "Muscle as an endocrine organ: Focus on muscle-derived interleukin-6." *Physiol Rev* 88: 1379-1406, 2008.

Unpublihed observations

## Interleukin-6



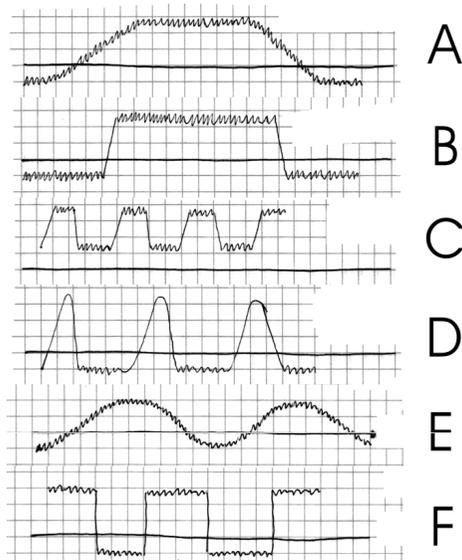
IL-6 is secreted by T cells and macrophages to stimulate immune response, i.e. during infection and after trauma especially burns or other tissue damage leading to inflammation.



## Interleukin-6: Pro- or anti-inflammatory?

### IL-6 acts as both a pro-inflammatory cytokine and anti-inflammatory myokine

#### Cytokine



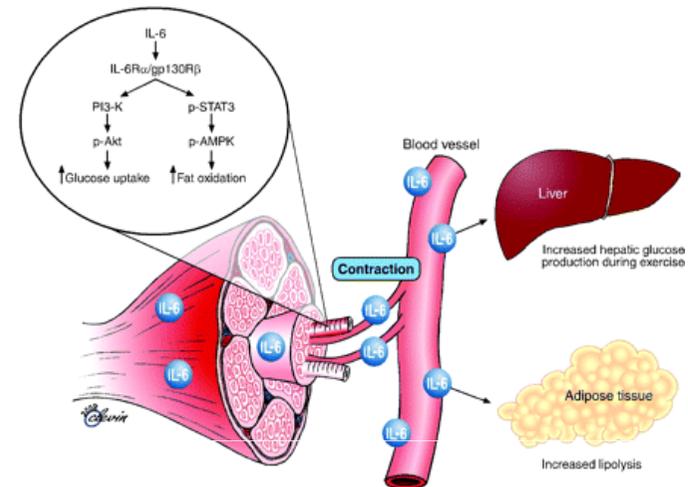
A  
B  
C  
D  
E  
F

Performance of the various types of fever

- a) Fever continues
- b) Fever continues to abrupt onset and remission
- c) Fever remittent
- d) Intermittent fever
- e) Undulant fever
- f) Relapsing fever

Mediator of fever - and of the acute phase responses

#### Myokine



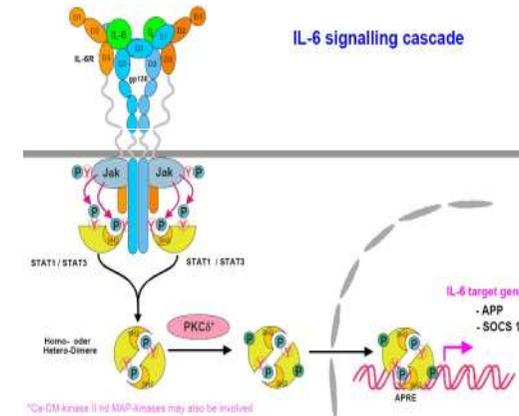
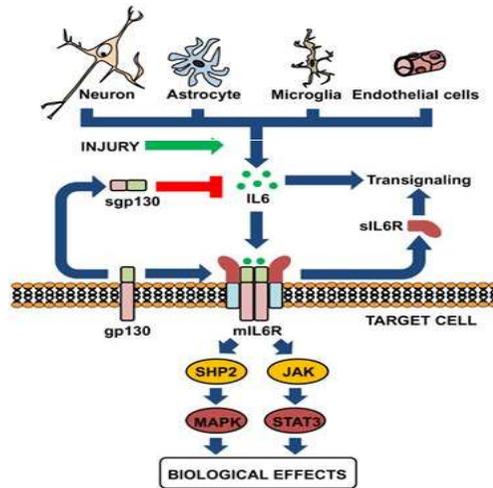
Mobilize extracellular substrates and/or augment substrate delivery

# IL-6 acts as both a pro-inflammatory cytokine and anti-inflammatory myokine

Macrophages/Monocytes

versus

Myocytes



## IL-6 signalling

The NFκB signalling pathway

TNF response

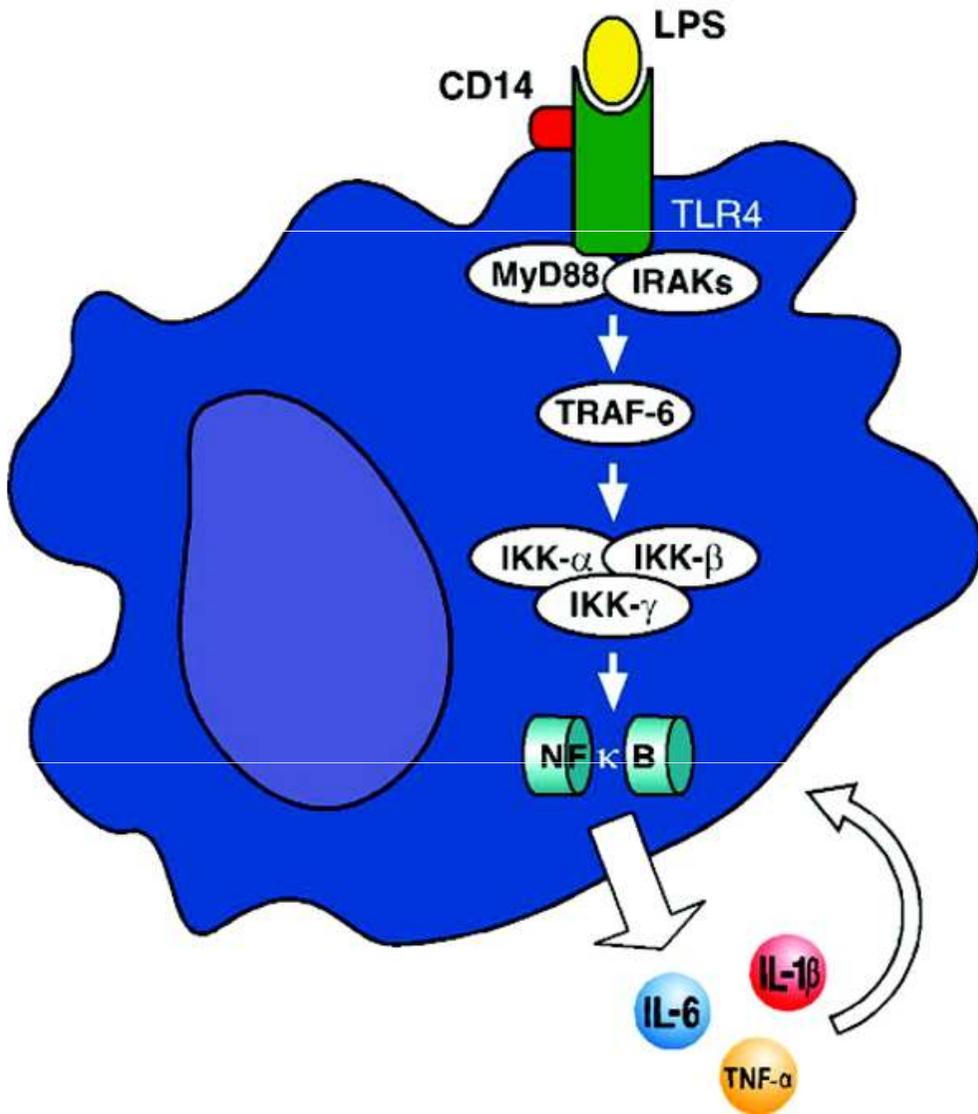
Pro-inflammatory - Cytokine  
Trauma

A network of signalling cascades, including the Ca<sup>2+</sup>/NFAT and glycogen/p38 MAPK pathways

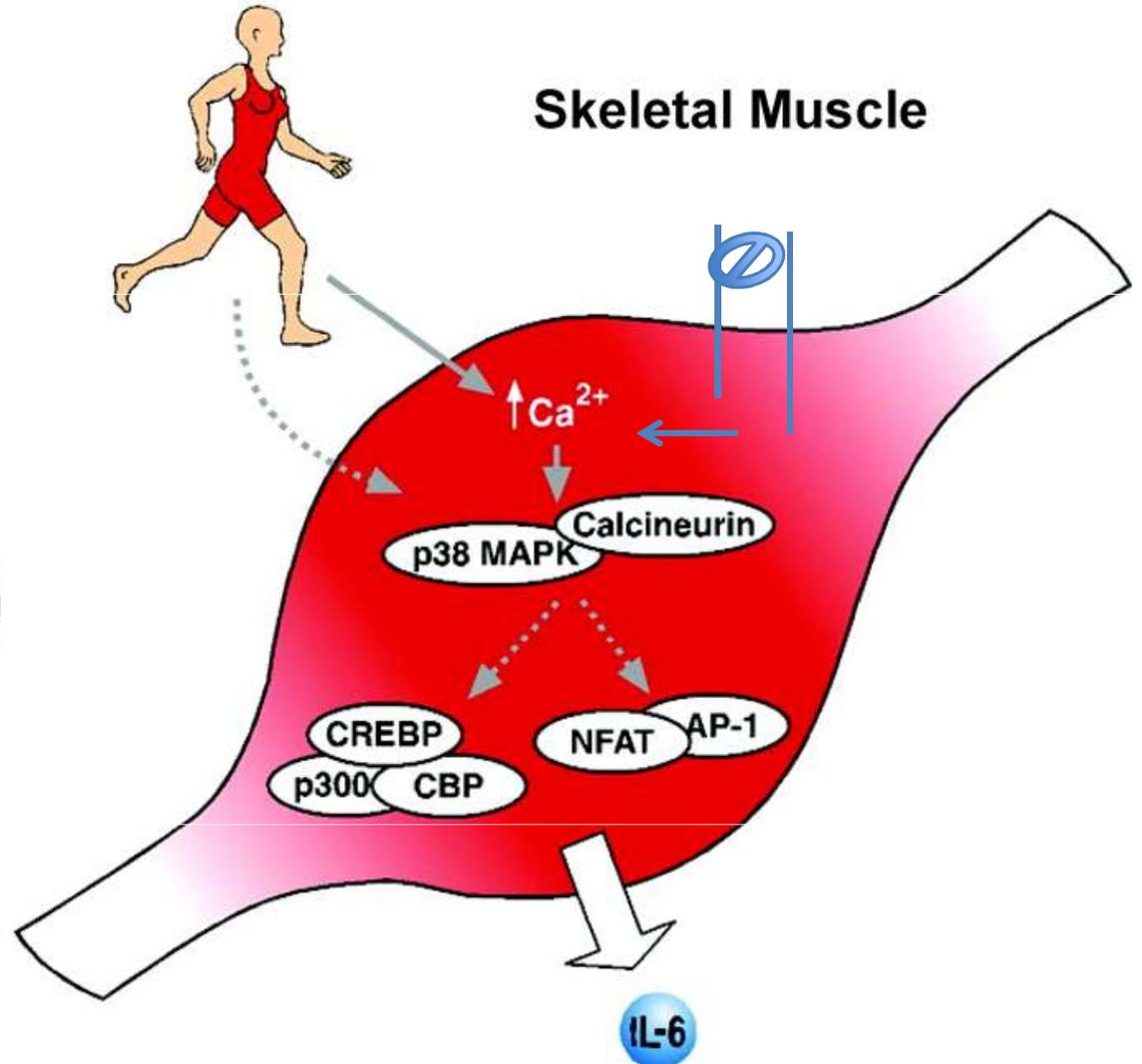
Anti-inflammatory – Myokine  
Exercise

# Overview

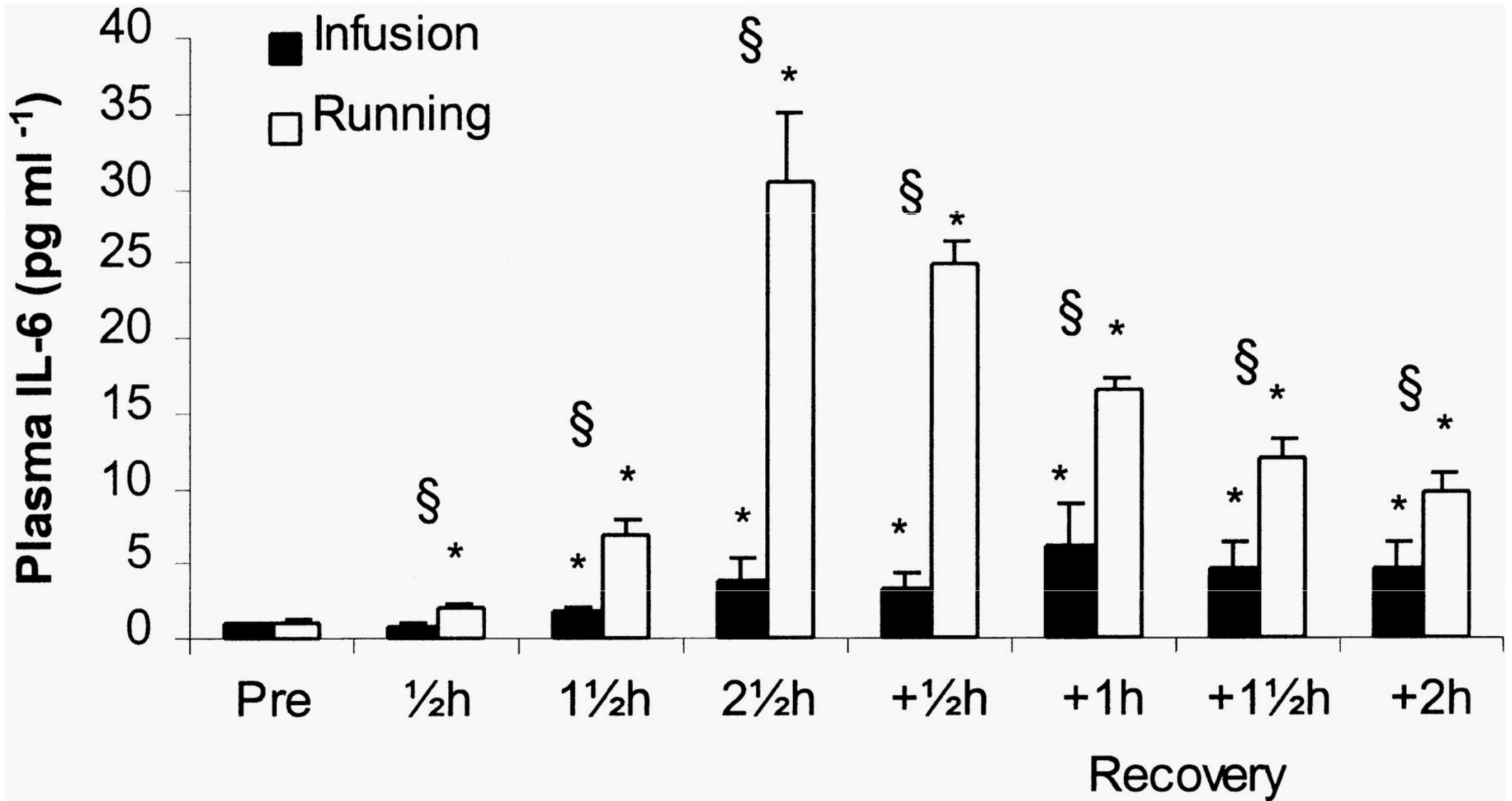
## Macrophage



## Skeletal Muscle

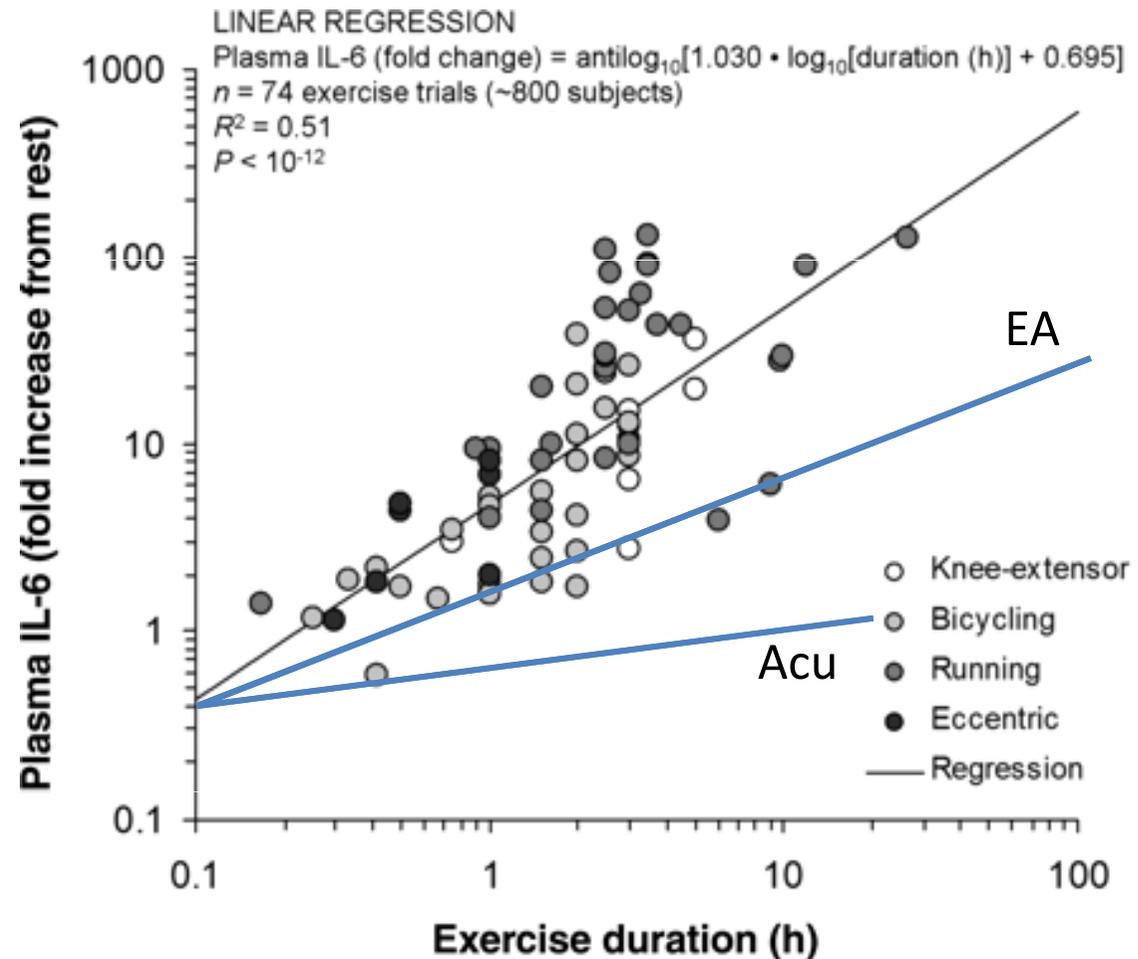


# Interleukin-6: Exercise and Epinephrine



## Interleukin-6 and Exercise

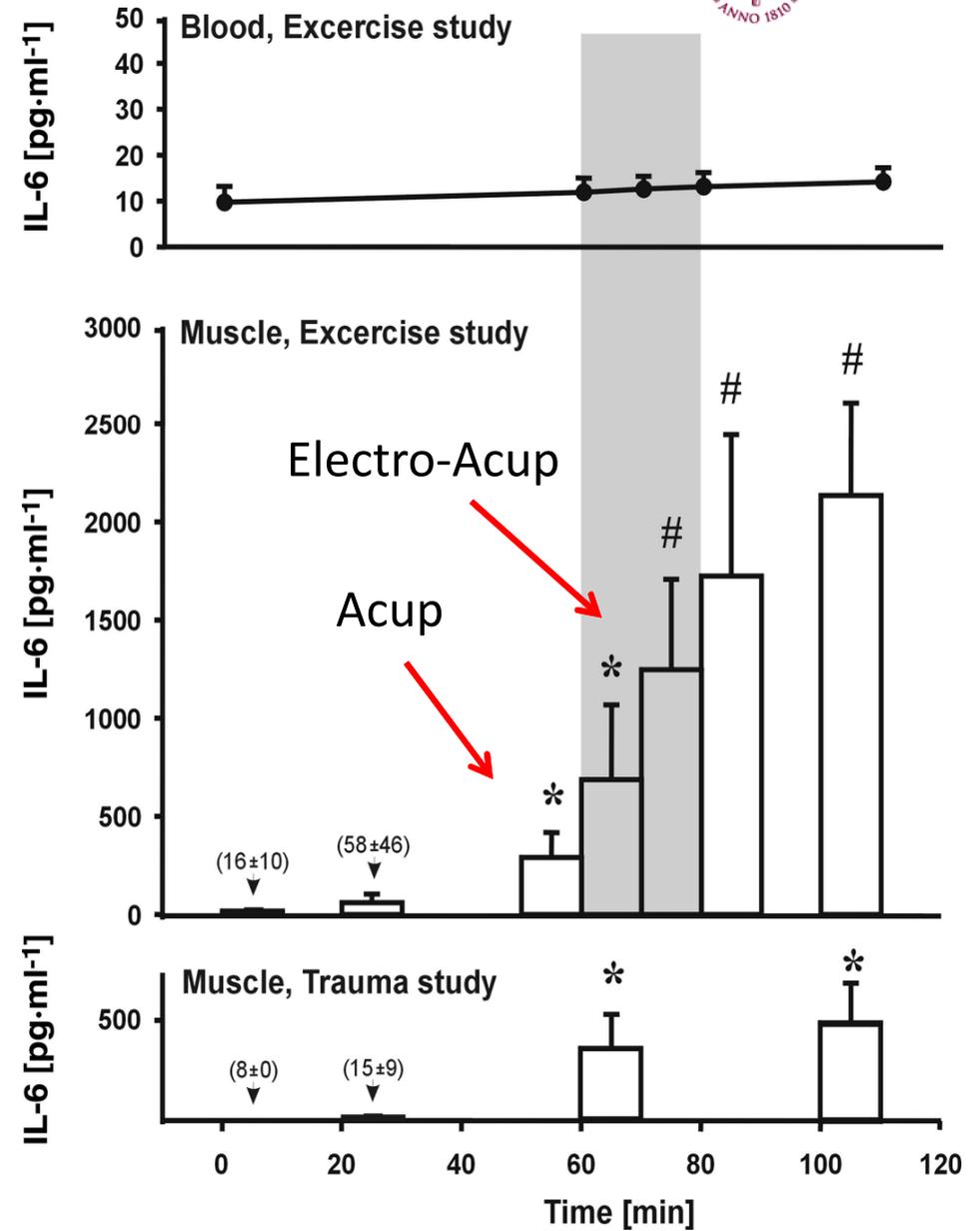
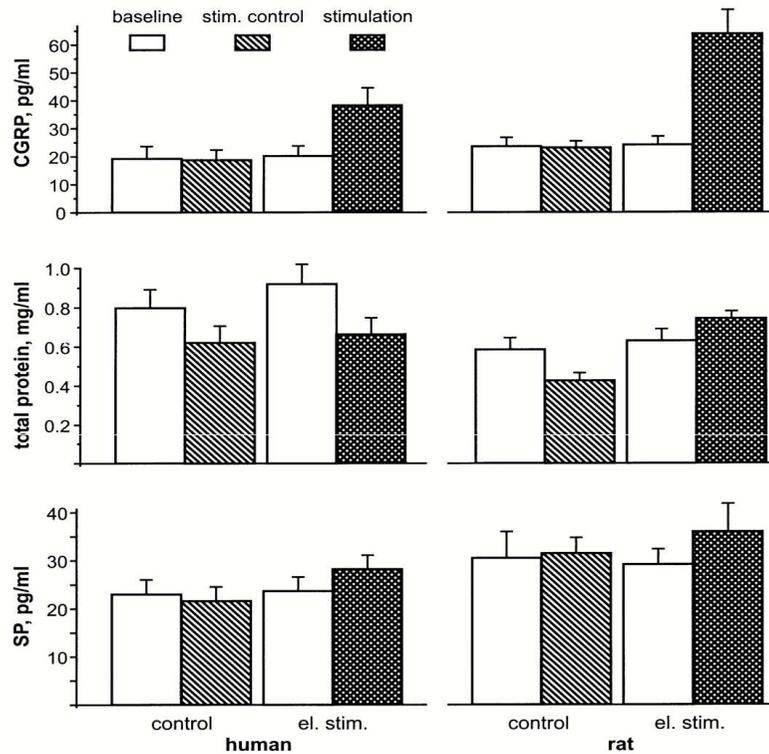
The overall log<sub>10</sub>-log<sub>10</sub> linear relation (straight solid line) between exercise duration and increase in plasma IL-6 (fold change from pre-exercise level) indicates that 51% of the variation in plasma IL-6 increase can be explained by the duration of exercise.



# Interleukin-6 and Exercise and Acupuncture

Manual acupuncture =  
Insertion of microdialysis probe

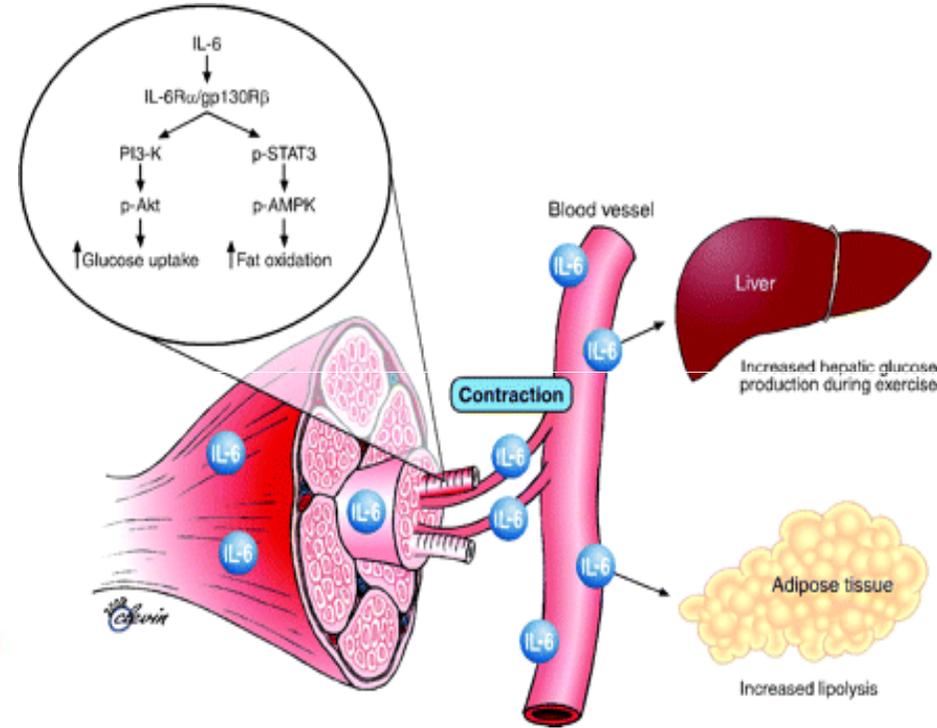
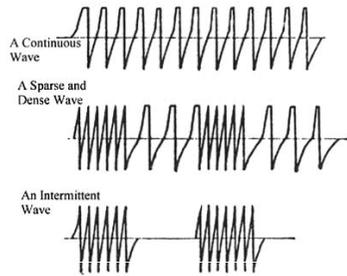
2Hz burst train Electro-Acupuncture =  
Exercise



Rosendal et al. Increases in interstitial interleukin-6 of human skeletal muscle with repetitive low- force exercise . J Appl Physiol 2005;98: 477-481

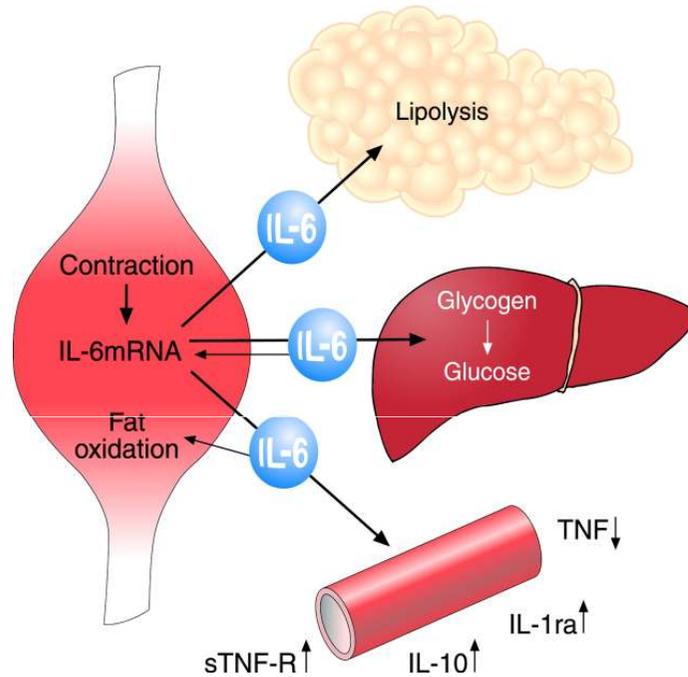
Lundeberg et al Increases in interleukin-6 and calcitonin gene-related peptide of human skeletal muscle following low force exercise and electro-acupuncture – unpublished observations

# IL-6 secreted into the role blood stream in response to muscle contraction:



## 1. Electro-acupuncture

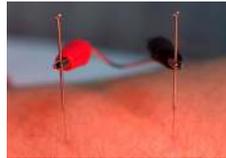
## 2. Exercise



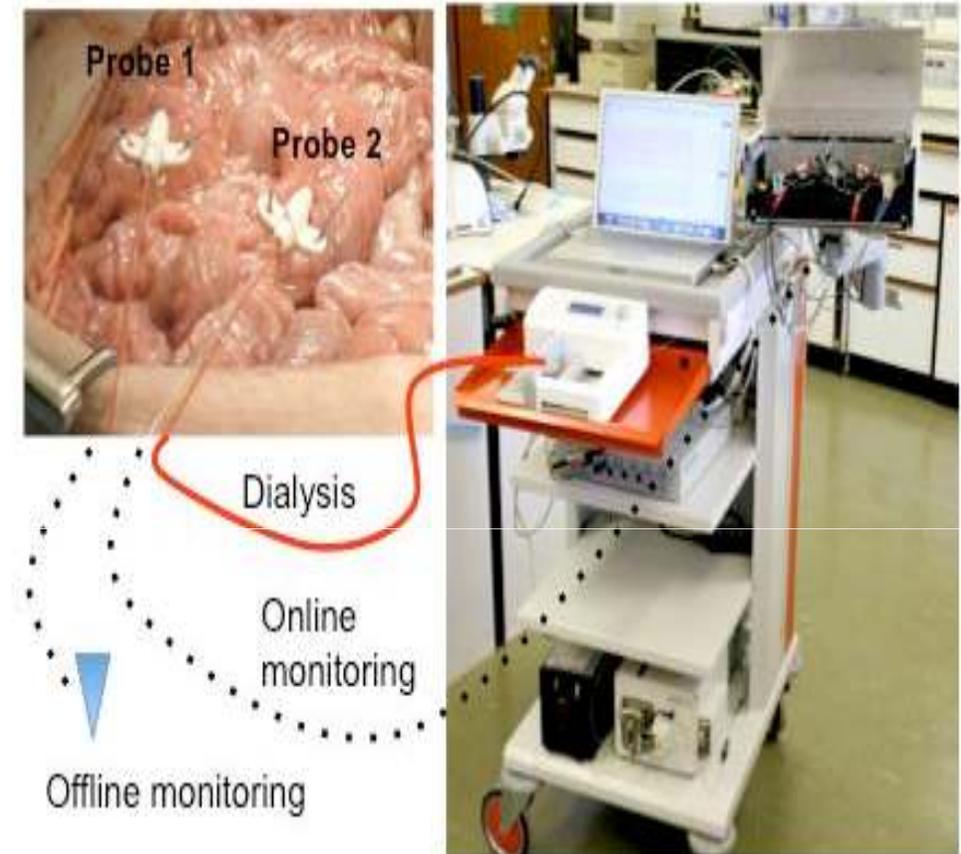
Pedersen BK, Fischer CP. Beneficial health effects of exercise: the role of IL-6 as a myokine. *Trends Pharmacol Sci* 28: 152–156, 2007.

Iizuka K, Machida T, Hirafuji M. Skeletal muscle is an endocrine organ. *J Pharmacol Sci*. 2014 Jun 19;125(2):125-31.

# Myokines under investigation showing altered levels during microdialysis during and after Exercise and Electro-acupuncture

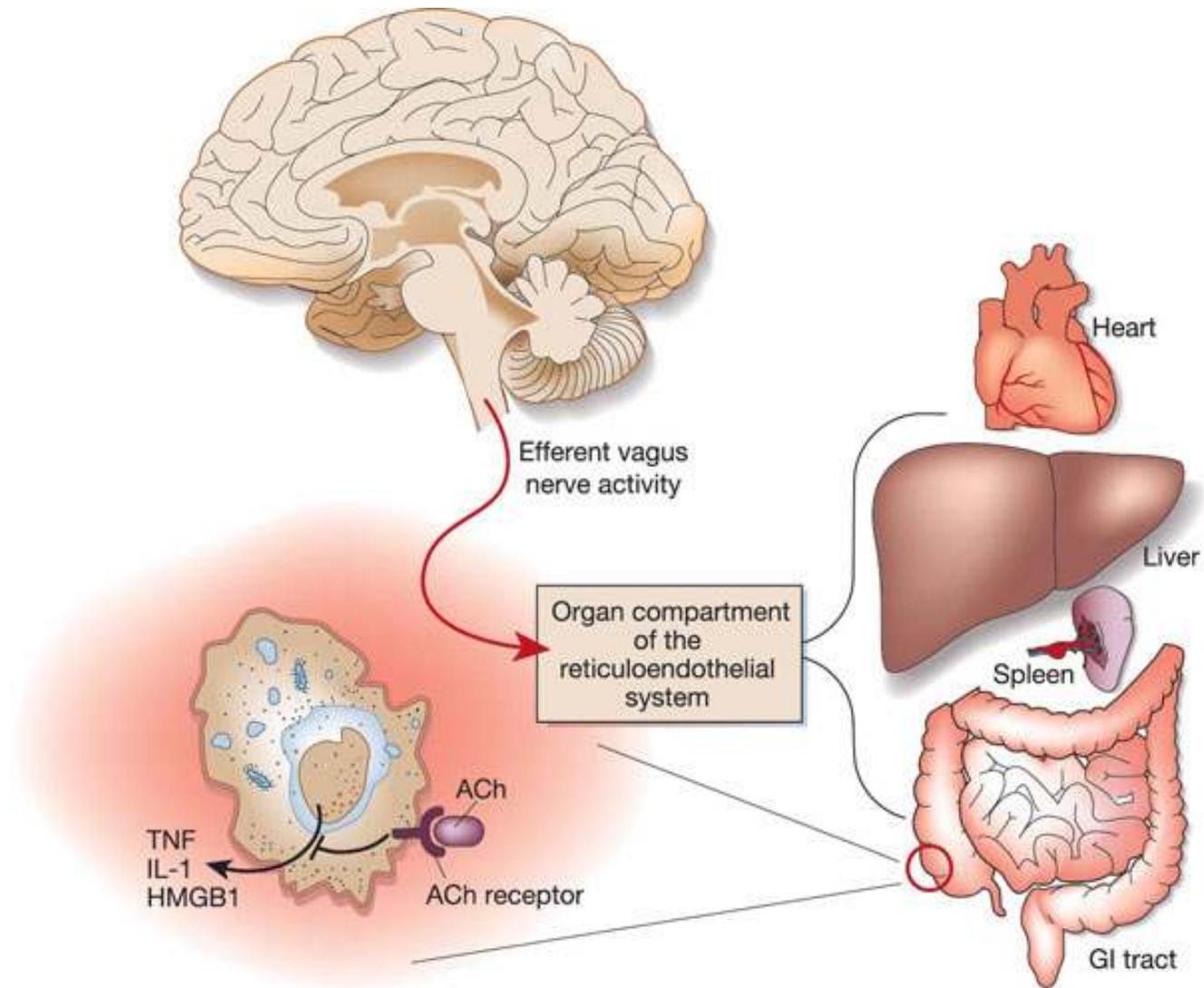


- Interleukin 15
- Brain-derived neurotrophic factor (BDNF)
- Myonectin
- Decorin
- Irisin
- Secreted protein acidic and rich in cysteine (SPARC)

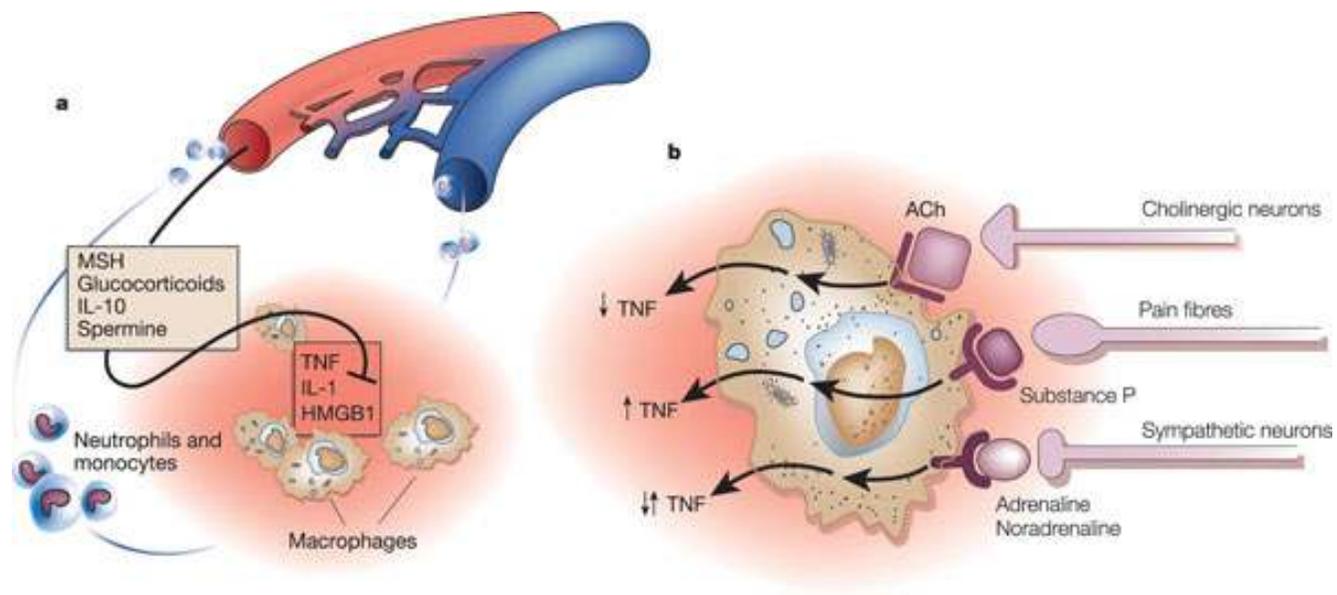


The immunomodulatory role of acupuncture may be dependent on more than direct peripheral modulation of nociceptors and high threshold receptors.

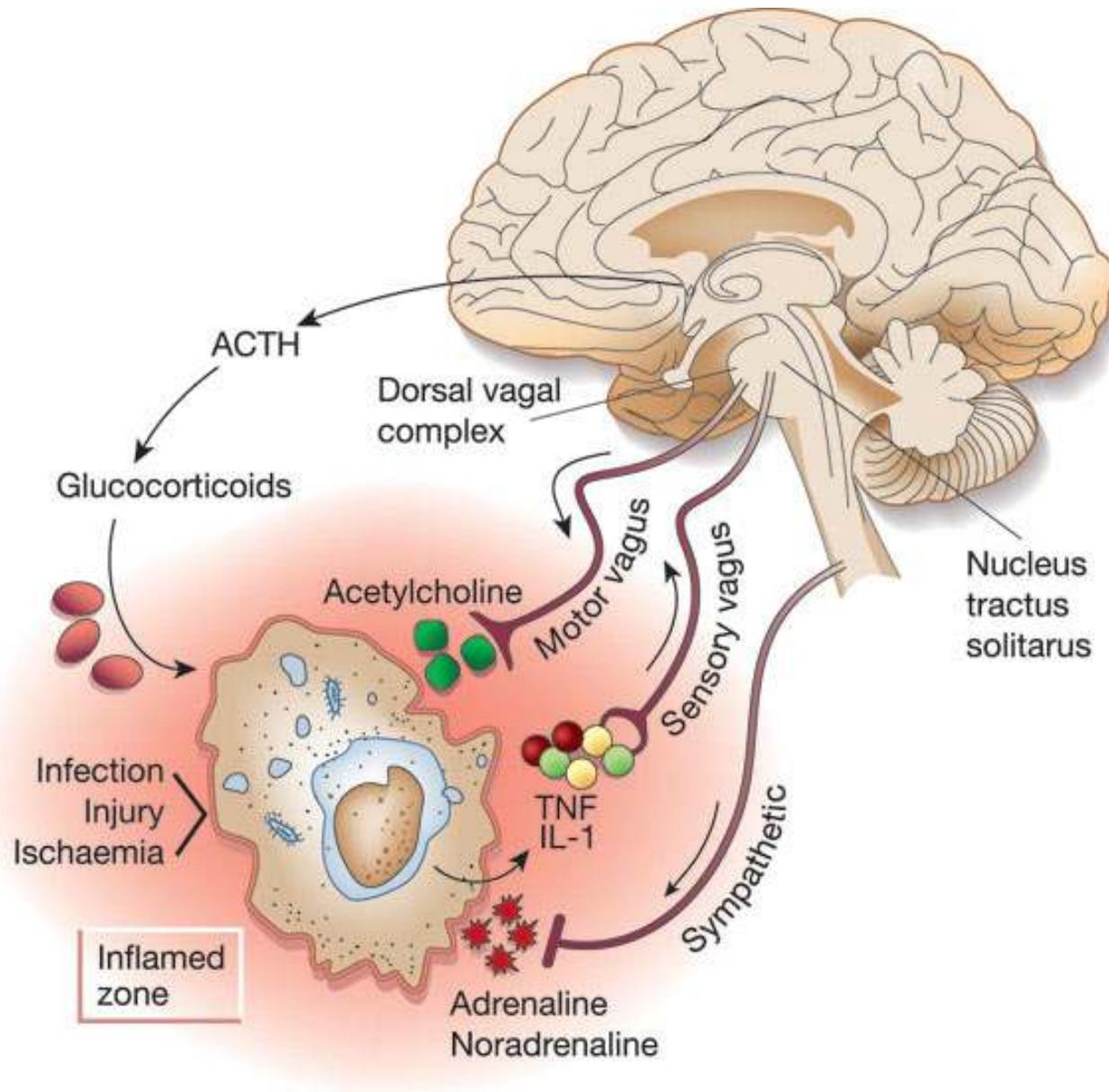
1. Spinal reflexes – increased sympathetic tone resulting in the release of immune cells from lymph glands
2. Vagal reflexes
3. Activation of the HPA-axis
4. Deactivation of limbic structures
5. Restoration of the default mode (allostasis-homeostasis)



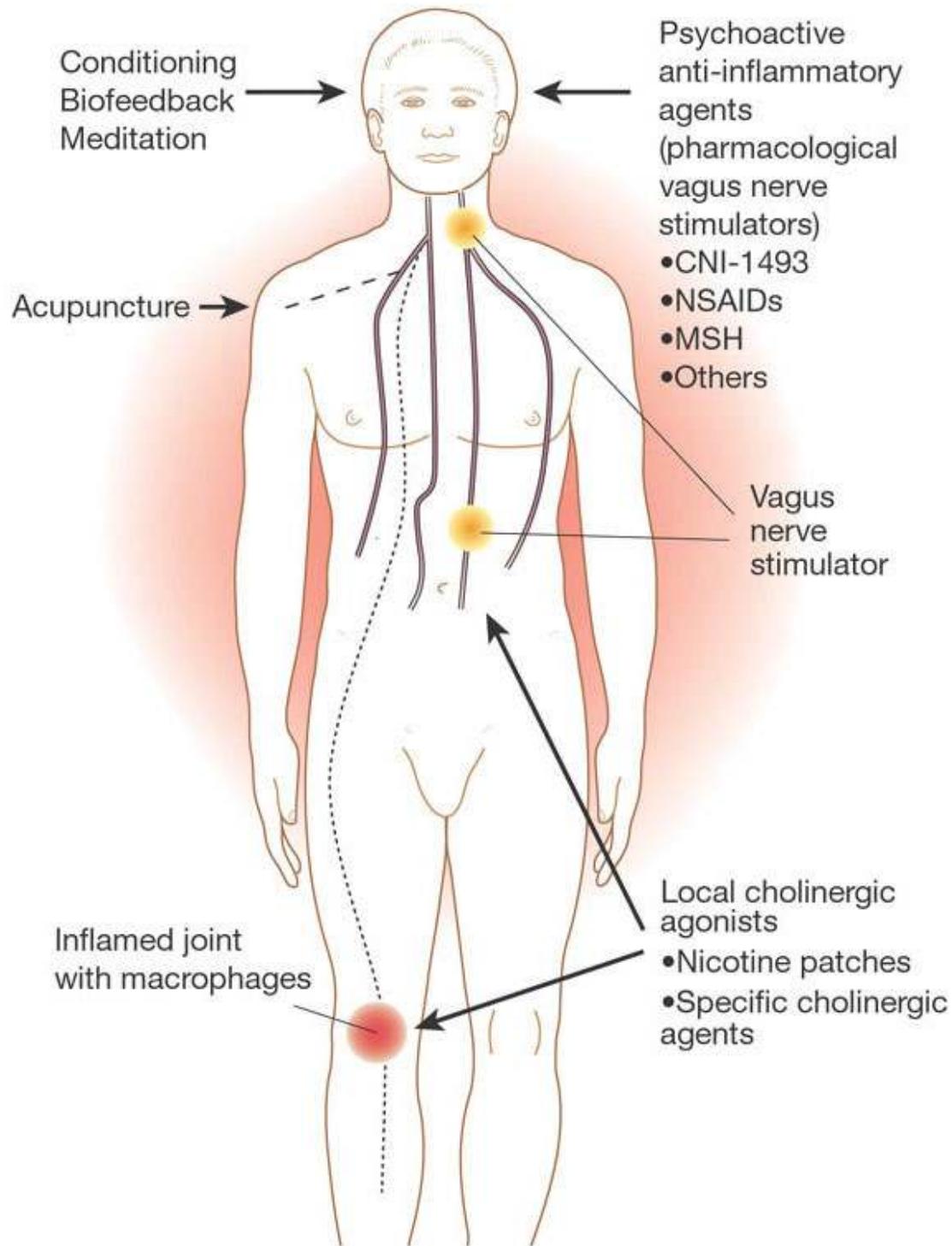
**FIGURE 1. The cholinergic anti-inflammatory pathway.**



**FIGURE 2. Diffusable versus neural anti-inflammatory pathways.**



**FIGURE 3. Wiring of the inflammatory reflex.**



**FIGURE 4. Targeting therapies to the cholinergic anti-inflammatory pathway.**



Tack så mycket