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Surgical Denervation of Specific Cutaneous Nerves Impedes Excisional Wound Healing of Small Animal Ear Pinnae

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Abstract

Damage to cutaneous nerves inhibits wound healing in patients. Results from animals on the nerve contributions to healing are various and sometimes contradictory. Here we aim to clearly define the collective role of central, caudal and rostral nerves in ear wound healing of mice, rats, and rabbits. These wounds heal with minimal contraction like wounds in humans. We resected central, caudal and rostral nerves at the base of ear pinnae by microsurgery, and created excisional full-thickness skin wounds in the pinnae neurologically downstream from the resections. Denervation in mice resulted in no closure for 14 days post-wounding (dpw) and led to only 17.2% closure at 21 dpw when the excisional wounds of non-denervated ear pinnae were completely closed. Compared to excisional wounds that were not denervated in sham surgery, wounds with denervation showed an increase of excisional wound areas for 5.0% by 7 dpw and a 43.7% reduction of wound closure at 12 dpw for rats. In rabbits, denervation attenuated wound closure for 14.2%, 34.4%, and 28.3% at 7, 14, and 18 dpw, respectively. Our histological analysis showed marked denervation impairment in pivotal healing processes, re-epithelialization and granulation tissue growth, suggesting denervation impairment in the regeneration of blood capillaries and/or connective tissue in wounds. These results reveal the critical contributions made by central, caudal and rostral nerves in ear pinnae to minimal-contraction skin wound healing. Our study also provides small animal models of minimal-contraction wound healing of denervated ear skins that recapitulate human wound healing involving surgical or traumatic nerve damages.

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Compliance with Ethical Standards Studies were conducted in conformity with the Public Health Service Policy on Humane Care and Use of Laboratory. Procedures using animals were approved by the Institutional Animal Care and Use Committee of Louisiana State University Health Sciences Center, New Orleans.

Conflict of Interest The authors declare that they have no competing interests.

Keywords

denervation; central; rostral; and caudal nerves in ear pinna; wound healing; epithelialization; microsurgery; small animal model

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Introduction

Millions of patients suffer from delayed wound healing. Wound healing in adult humans can be inhibited by damage to cutaneous nerves, such as through trauma or surgical procedures [1–4]. The interaction between skin and peripheral nerves is believed to contribute to wound healing [5]. There is increasing evidence that cutaneous innervation is an important modulator of the normal wound healing process. Following damage caused by wounds, tissue develops a dense nerve network and forms close associations with cells within the wound environment, including endothelial cells [6], macrophages [7] and myofibroblasts [8]. Nerve systems produce reparative neuropeptides, regulate blood flow, and modulate neurogenic inflammation [9, 10], and local immune responses [11, 12], all of which contributes to wound healing. The loss of neural function in the injury site could delay wound healing, leading to chronic wounds [13, 2].

There are several studies where denervation was observed in wound models, leading to impaired wound healing [14, 15], reduced wound contraction, epithelization, or granulation [2]. However, these conclusions were drawn from experiments on rodent dorsal skin wounds that are mainly healed by contraction, whereas human wounds are healed by re-epithelialization [16–18]. Furthermore, the nerves denervated by capsaicin or surgical procedures were not clearly defined, and capsaicin has significant side effects [13, 2, 6]. Recently, Ferguson's group found that comprehensive denervation of mouse ear pinnae resulted in impairment of outer ear regeneration from punched through-and-thorough holes [19]. However the muscles and cartilage underneath the skin were removed in the punched through-and-thorough holes, which may not recapitulate common human skin wounds with muscles and bones underneath. Moreover, the comprehensive denervation may be difficult to reproduce quantitatively on many animals in the mechanistic studies because it demands great skill and much time. Veves' group found that excisional wounds, generated on rabbit ear pinnae that were made in neuroischemic [ligation of (central + rostral arteries) + resection of (central + rostral nerves)] or ischemic animals [ligation of (central + rostral arteries)] closed less when compared to sham animals (without ligation or resection). The major nerves entering the ear pinnae from the bases are central, caudal and rostral nerves. Unlike Ferguson's group, which punched through the pinnae as well as the cartilage, Veves' team kept the cartilage intact since it stents the wound open and minimizes tissue contracture to less than 3%, allowing the wound to heal by re-epithelialization instead of contraction, thus recapitulating the healing found in human skin wounds [20]. However, the closure rates for neuroischemic and ischemic wounds were not significantly different, suggesting that the resection of central and rostral nerves might not be enough to affect wound closure. More nerves may need to be denervated in order to demonstrate the nerve's role in wound closure.

The background research above motivated us to conduct the experiments described in this report. Using mice, rats and rabbits, we tested the hypothesis that the central, caudal and rostral nerves of ear pinnae critically contribute to the healing of contraction-minimal pinna skin wounds. The caudal nerves were resected too, in addition to the central and rostral nerves. Using these small animals we just mentioned, we studied the full-thickness excisional skin wounds of surgically-denervated ear pinnae with intact cartilage underneath to minimize the wounded skin contraction. The wound closure, re-epithelialization, and granulation tissue growth were found to be markedly attenuated by the denervation. Moreover, the wound bed tissue and underlying cartilage in the pinnae regressed and formed holes in the denervated mice, but not in rats, rabbits or non-denervated mice. Taken together, the results of our experiments supported our hypothesis, and thus we were able to determine the species-dependent differences in the collective role of select nerves in contraction-minimal wound healing and preliminarily establish the models using these animals.

Materials and methods

Studies were conducted in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory. Procedures using animals were approved by the Institutional Animal Care and Use Committee of Louisiana State University Health Sciences Center, New Orleans. We used mice, rats and rabbits that are detailed in the experiments below.

Surgical procedures for ear pinna denervation and skin excision

To determine roles of cutaneous nerves in skin wound healing, we resected the key nerve supplies to the left ear pinnae of mice, rats, and rabbits before wounding the denervated areas. This was conducted based on established neuroanatomy [21–23]. All surgical procedures were performed under anesthesia to access the rostral, caudal and central auricular nerves in the ears of mice, rats and rabbits. During surgical process, we used an operating microscope and microsurgical scissors for precision cutting of the auricular nerves without damaging the surrounding tissues and blood vessels. The details of the surgical procedures are described below for mice, rats and rabbits. After we established the surgical procedures, we acquired the quantitative data for each surgical condition (denervation or sham surgery without denervation) at each time point from four animals of each species (mice, rats, or rabbits).

(A) Microsurgical denervation and full-thickness skin excision of mouse and rat ear pinnae

C57BL/6 mice or Sprague-Dawley rats (female, 8–10 weeks of age, Charles River Lab) were anesthetized by intraperitoneal injection of ketamine hydrochloride and xylazine (10 mg/kg body weight) according to the standard protocol proposed by the animal facilities of the Louisiana State University Health Sciences Center, New Orleans. Under appropriate anesthesia, the hair in the surgical areas of the ear pinnae was depilated using Veet hair removal cream, and the skin surfaces for surgery were sterilized with 70% ethanol. We injected sterile saline (100 µl per site) intra-dermally into the pinna bases of the left ears to generate pouches. We then made an incision (8 mm for mice or 10 mm for rats) through the pouch using a No. 11 scalpel with a triangular blade to access the auricular nerves. Select auricular nerves (central, caudal and rostral) were picked out from surrounding tissues with

microsurgical forceps and resected 2–3 or 4–5 mm length, for mice or rats, respectively, with microsurgical scissors without damaging the surrounding tissues and blood vessels. Immediately following the microsurgical procedures, 2-mm-diameter circular skin in full-thickness was excised from the left ear pinna above the base where the nerves were resected. Each excision was made using a 2-mm biopsy punch and microforceps. The same procedure was performed in the contralateral control ear on the right without the denervation. All the surgical procedures were performed carefully without damaging the auricle cartilage. The skin incisions were closed with 8-0 non-absorbable prolene sutures. The wounds were finally covered with transparent film dressings.

(B) Microsurgical denervation and full-thickness skin excision of rabbit ear pinnae

New Zealand white rabbits (~2.5 kg, 2.5 months of age, female, Charles River Lab) were anesthetized by intramuscular (epaxial muscles) injection of ketamine hydrochloride and xylazine (80 mg/kg body weight), a standard dosage posted by our animal facilities. Procedures similar to those of mice were conducted. Briefly, the surgical areas of the ear pinnae were depilated and sterilized. Sterile saline (200 µl per site) was injected intradermally into pinna bases of left ears to generate pouches, and then 15 mm incisions were made through each pouch using the scalpel to expose the auricular nerves. Under the direct vision via an operating microscope, we picked out the key auricular nerves (central, caudal and rostral) from surrounding tissues with microsurgical forceps and resected them (3–5 mm length) using microsurgical scissors without damaging the surrounding tissues and blood vessels. The incisions were closed with 8-0 non-absorbable prolene sutures. We then made four 6-mm-diameter circular full-thickness excisions of the pinna skin neurologically downstream of the denervation site at the pinna bases of the left ears. Each excision was made by a 6-mm-diameter biopsy punch. The auricle cartilage was intact in the surgery. The procedures were also performed in the contralateral ear on the right without the denervation, which was used as the non-denervated control. Transparent film dressings were used to cover the wounds.

Due to the small diameter of the nerves in these small animals (mice, rats, and rabbits), denervation surgery was conducted using an operational microscope and other microsurgery tools. The saline pouches at the skin sites for the denervation surgery, *i.e.*, the bases of the ear pinnae, were needed to access these nerves. Denervation surgery of the mice consumed more time compared to rats or rabbits due to the animal's smaller size, and there are no previous publications that described this surgical denervation in rats.

Post-operative care

Each animal was housed in a separate cage after surgery. Animal behavior was monitored, including foraging, grooming, drinking, and eating. Sutures were removed from incisions at 5 days post-wounding (dpw).

Analysis of wound healing

Wound healing was measured according to our previously-conducted methods [24–29]. Briefly, each wound was photographed with a digital camera (Canon) alongside a thin ruler used as a graded scale bar. The wound areas in the photos were analyzed using NIH ImageJ

software. The wound closure was calculated as a percentage of closed wound area relative to the initial wound area at 0 dpw. The excisionally-wounded skin of the ear pinnae (rimmed with normal skin) were excised from animals after sacrifice; fixed in 4% paraformaldehyde for 12 hours, soaked in 30% sucrose overnight, and then embedded into OCT (cryo-preserved). Serial cryosections (10 μ m thick/section) of each wound specimen were conducted through the center region of the widest wound-bed on each wound specimen. The section of each wound representing the widest wound bed or the center of the wound were chosen for the following analysis. The epithelial gap and granulation tissue area were measured from the selected sections after hematoxylin and eosin (HE) staining. The HE stained images were captured using Olympus scanning microscope and software OlyVIA2.4. Adobe Photoshop 9.0 software was used to measuring re-epithelization (%) and granulation area (mm²). Re-epithelialization percentage was calculated as [(wound diameter at 0 dpw – epithelial gap (distance between advancing edges of migrated keratinocytes in the wound cross-section))/(wound diameter at 0 dpw) $\times$ 100%] as previously described. Granulation tissue was new connective tissue and blood vessel capillaries growing from the base of the wound [29].

Statistical analysis

All data were presented in mean $\pm$ standard deviation (SD), and analyzed using either one-way or two-way ANOVA tests. A *p*-value of < 0.01 or < 0.05 was considered statistically significant. SPSS software (www.ibm.com) was used for the statistical analysis.

Results

Development of surgical procedures to resect select nerves entering ear pinnae of mice, rats and rabbits

The central, caudal, and rostral auricular nerves that enter the ear pinnae of these small animals were selected for the denervation study because they are the key nerve supplies to the downstream skin area of the ear pinnae [21–23]. These nerves are thin (especially for mice), and surrounded by arteries, veins, and other tissues. In order to identify the nerves, the denervation surgery was performed under an operating microscope; we also generated saline pouches in the skins by injecting saline intradermally to the base of ear pinnae (100 μ l/ear for mice and rats, and 200 μ l/ear for rabbits). Ferguson's group also used saline pouches in their denervation operation of mouse ears [19]. We found that without the pouch, it is difficult to access the nerves of these small animals, especially for mice. To separate nerves from surrounding tissues, including arterials and veins, we mainly used microforceps. When cutting the connecting tissues with scalpels or microscissors, we checked the tissue from different angles to ensure the blood vessels were not damaged. The damage to blood vessels at the base of ear pinnae reduces healing of the excisional wounds made to the downstream skin areas following the denervation, which could confound the outcome of the denervation study. After the animals were sacrificed, the previously-incised skin at the ear bases were re-incised and the resection sites were examined; there was no evidence of reconnection of the resected nerves.

Surgical denervation of select nerves markedly delayed the closure of full-thickness skin excision wounds of mouse, rat and rabbit ear pinnae

To clearly identify the collective role of typical nerves (central, caudal and rostral nerves) of ear pinnae in wound healing, and to develop the small animal models for the study targeting this role, we first determined the closure time courses of wounds in the surgically-denervated skin of the ear pinnae. A 2-mm-diameter excision of full-thickness skin was imposed to an ear pinna of C57BL/6 mice (Fig. 1) or Sprague-Dawley rats (Supplemental Figure 1) with (left ear) or without (right ear) the surgical denervation. In parallel, four 6-mm-diameter excisions of full-thickness skin were created in each ear pinna of New Zealand white rabbits (Figures 3 and 4) with or without the surgical denervation. The non-denervated pinna excisions of mice, rats or rabbits had minimal apparent necrosis and shed scabs from ~5 dpw. The denervation of mouse ear pinnae resulted not only in no closure for 14 days, but also tissue regression in the wound bed (Fig. 1a). Specifically, tissues (including cartilages under the wound bed) were completely lost by 6 dpw, resulting in a hole that penetrated through the ear at 2-mm diameter, the size of the original excision. The hole size expanded 3.1% ($\pm 2.9\%$, $n = 4$) by 14 dpw.

Tissue along the edges of the holes grew back slowly. By 21 dpw, the holes closed only 17.2% ($\pm 2.1\%$, $n = 4$, $P < 0.01$ vs 0% closure at 0 dpw), whereas the excisional wounds of non-denervated ear pinnae were completely closed ($\pm 0\%$, $n = 4$, $p < 0.01$ vs 17.2% $\pm 2.1\%$ closure of denervated wounds at 21 dpw) (Fig. 1b). It is interesting that the excisional wounds of non-denervated mouse ear pinnae [on the contralateral (right) side of the animal] did not form piercing holes; they closed 21.9% ($\pm 3.4\%$, $n = 4$, $p < 0.01$ vs 0% for denervated wounds at 6 dpw) by 6 dpw and 77.3% ($\pm 2.3\%$, $n = 4$, $p < 0.01$ vs 0% for denervated wounds at 14 dpw) by 14 dpw. In contrast to mice, the denervation did not cause the formation of piercing holes in rats (Fig. 2) or rabbits (Fig. 3). For rats, the denervation led to a 5.0% ($\pm 2.5\%$, $n = 4$) increase of excisional wound areas by 7 dpw; and 43.7% reduction of wound closure at 12 dpw compared to non-denervated wounds [56.3 $\pm 5.1\%$ vs 100 $\pm 0\%$ (completely closed), $n = 4$, $p < 0.01$] (Fig. 2b). For rabbits (Fig. 3), the results of non-denervated wound closures (%) minus denervated wound closures (%) are 14.2% (14.2 $\pm 2.1\%$ vs 0%, $n = 4$, $p < 0.01$), 34.4% (63.2 $\pm 3.9\%$ vs 28.8 $\pm 5.0\%$, $n = 4$, $p < 0.01$), and 28.3% (76.7 $\pm 4.2\%$ vs 48.4 $\pm 5.4\%$, $n = 4$, $p < 0.01$) at 7, 14, and 18 dpw, respectively. Thus the surgical denervation of central, caudal and rostral nerves delayed closure of full-thickness skin excision wounds of mouse, rat and rabbit ear pinnae.

Surgical denervation of central, caudal and rostral nerves reduced re-epithelialization and granulation tissue formation of wounds in ear pinnae

Re-epithelialization and granulation tissue growth are pivotal processes of skin wound healing. When the central, caudal, and rostral nerves were resected at the bases of the ear pinnae, the excisional wounds of pinna skins (where the nerves entered) manifested significantly lower re-epithelialization and granulation tissue formation (Fig. 4). For rats, re-epithelialization and the granulation tissue area of denervated wounds were 20.8% [79.2 $\pm 8.7\%$ vs 100 $\pm 0.0\%$ (completely closed wounds), $n = 4$, $p < 0.05$] and 16.4 mm² (38.9 ± 2.1 mm² vs 55.3 ± 2.3 mm², $n = 4$, $p < 0.01$) less compared to non-denervated wounds at 12 dpw (Fig. 4b). For rabbits (Fig. 4c), the re-epithelialization of denervated wounds were

8.9% ($58.0 \pm 3.7\%$ vs $66.9 \pm 3.3\%$, $n = 4$, $p < 0.05$) less than the non-denervated wounds at 7 dpw, but the difference disappeared at 18 dpw. The granulation tissue areas of denervated wounds (Fig. 4c right) were 7.2 mm^2 ($38.5 \pm 1.6 \text{ mm}^2$ vs $45.7 \pm 1.3 \text{ mm}^2$, $n = 4$, $p < 0.05$) less at 7 dpw and 8.0 mm^2 ($27.7 \pm 1.4 \text{ mm}^2$ vs $35.7 \pm 1.1 \text{ mm}^2$, $n = 4$, $p < 0.01$) less at 18 dpw compared to non-denervated wounds. It is noticed that the denervation attenuated wound closure of rabbits by 28.3% at 18 dpw (Fig. 3) whereas the re-epithelialization completely covered the wound epithelial gap for both denervated and non-denervated wounds (Fig. 4c left). This is because the scab area covering the immature newly-grown epidermis was taken as unclosed wound area by the standard measurement of wound closure (Fig. 3) [16–18]. This wound closure may represent the healing status better since the immature newly-grown epithelia are likely unable to provide the needed mechanical protection and barrier against water evaporation or leakage. Most of the animals (~95%) that underwent the denervation and excisional wounding survived until sacrificed and resumed normal eating, grooming, and movement 12 hours after the surgery.

Discussion

There is compiling evidence demonstrating that nerves significantly contribute to adult human skin wound healing [1–4]. However, reported experimental evidence is lacking so far for the role of central, caudal, and rostral nerves of ear pinnae in ear tissue maintenance and regeneration. The scarcity of this knowledge not only affects studies of modalities and mechanisms in ear regeneration, but also limits the usage of excisional ear-skin wound models, which can recapitulate the contraction-minimal healing process of human wounds, in the studies of mechanisms and therapeutic development involving the role of nerves in human skin wound healing. We here aimed to fill the holes of this important gap of knowledge. Several nerves enter ear pinnae from their bases. We conducted specific denervation of the ear pinnae by surgically resecting the central, caudal and rostral nerves because they are likely to play a critical role in ear wound healing [18] and they can be reproducibly accessed and resected.

Denervation of the central, caudal, and rostral nerves profoundly impeded the healing of full-thickness skin wounds created in the ear pinnae where these nerves enter (Figs. 1–4). The impairment was stronger in mice than that in rats or rabbits. By 6 dpw in mice, the denervation resulted in regression of the wound bed tissues and formation of a hole penetrating through the ear to the backside. The tissues along the edges of the holes grew back slowly (Figs 1 and 2). This kind of tissue regression did not occur in rats or rabbits, suggesting the denervation-associated tissue regression is species-dependent. The denervation-associated regression or regeneration of tissues, including ear cartilage in mouse ear pinnae, could be used as a mouse model in the study of nerve-involved tissue maintenance and regeneration.

Surgical denervation impaired closure of the excisional full-thickness skin wounds of the ear pinnae of mice, rats, and rabbits (Figs. 1–3). There was more reduction of the wound closure in mice compared to rats or rabbits. The denervation also impeded re-epithelialization and granulation tissue growth of excisional wounds of the ear pinnae, both of which are pivotal processes of wound healing. The reduced granulation tissue growth indicates impairment of

the regeneration of connective tissues and/or blood vessel capillaries that constitute the granulation tissues, and which are critical in wound healing.

We noticed that the excisional wound healing of the small-animal ear pinnae was markedly slower compared to the excisional wound healing of the dorsal skin of C57BL/6 mice. Our previous experiments showed that the 4-mm diameter wounds of full-thickness dorsal skin, which had contraction diminished by splints, need 14 days to close completely [30], whereas in this report the 2-mm diameter ear wounds of the same type of mice without denervation needed 21 days for 100% closure to occur (Fig. 1). The mechanism underlined this difference in closure rate warrants future studies.

There are multiple potential mechanisms that are likely to be responsible the denervation impairment on ear excisional wound healing that was observed in this report. Cutaneous nerves innervate in the epidermis, dermis, and hypodermis, and some of these nerves terminate in free nerve endings. These nerves contribute to the healing of skin wounds, including ear pinna wounds. Innervation is likely to play a pivotal role in healing by regulating wound cellularity [13]. Capsaicin induced denervation affects cellular mechanism in wound environment, including proliferation and apoptosis [13], reduced microvascular response and neovascularization; and prolonged inflammatory phase [2]. There were reduced monocyte, macrophage and T-lymphocyte counts in denervated wounds four days post-wounding [31], which is likely to contribute to the denervation impairment of wound healing. Denervation attenuates the levels of potent vasodilatory and chemotactic neurotrophins (including nerve growth factor) and neuropeptides, such as substance P, which triggers leukocyte chemotaxis, activation, and synthesis of transforming growth factor alpha. Substance P also induces expression of intracellular adhesion molecule-1 [31]; and calcitonin gene-related peptide, a neuropeptide found in C and Aδ sensory axons that stimulates T-lymphocyte and neutrophil migration [32]. Additionally denervation interrupts the intimate interactions between nerves (such as axons and or Schwann cells) and surrounding cells (such as keratinocytes, endothelial cells [6], fibroblasts [8], and macrophages [7]), which are pivotal for normal wound healing. There is still a lack of reliable animal models that replicate the effect of skin nerve damage to human wounds and that can be conducted easily. These models are the basis for further exploration of the mechanisms and therapies for wound healing. The small animal models preliminarily developed in this project provide a new useful tool to study wound healing.

Conclusion

To better recapitulate the role of nerves in adult human wound healing and find a better animal model for this purpose, we resected select nerves that supply the mouse, rat or rabbit ear pinnae at the entrance or base and conducted excisional wound healing of the denervated pinna skin. This denervation caused a marked decline in wound healing, including wound closure, re-epithelialization, and granulation tissue growth, indicating that the denervation impeded the regeneration of blood capillaries and/or connective tissue in wounds. Importantly, some species variation was observed.

Based on the results of this report, further studies are required to determine the role of nerves in blood vessel regrowth and function, inflammation and leukocyte infiltration, infection, collagen deposition and wound remodeling, effectiveness of the skin barrier against water loss, and the mechanisms underlining the role of nerves, including nerve-produced neurotrophins and neuropeptides. Additionally, the unique small-animal models developed or modified in this report are useful tools that can facilitate the studies of mechanisms and the therapeutic development of nerve-involved wound healing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Laverdet B, Danigo A, Girard D, Magy L, Demiot C, Desmouliere A. Skin innervation: important roles during normal and pathological cutaneous repair. *Histology and histopathology*. 2015; 30(8): 875–892. DOI: 10.14670/HH-11-610 [PubMed: 25799052]
2. Barker AR, Rosson GD, Dellon AL. Wound healing in denervated tissue. *Annals of plastic surgery*. 2006; 57(3):339–342. DOI: 10.1097/01.sap.0000221465.69826.b7 [PubMed: 16929207]
3. Ebenezer GJ, McArthur JC, Thomas D, Murinson B, Hauer P, Polydefkis M, Griffin JW. Denervation of skin in neuropathies: the sequence of axonal and Schwann cell changes in skin biopsies. *Brain*. 2007; 130(Pt 10):2703–2714. DOI: 10.1093/brain/awm199 [PubMed: 17898011]
4. Basson MD, Burney RE. Defective wound healing in patients with paraplegia and quadriplegia. *Surgery, gynecology & obstetrics*. 1982; 155(1):9–12.
5. Westerman RA, Carr RW, Delaney CA, Morris MJ, Roberts RG. The role of skin nociceptive afferent nerves in blister healing. *Clinical and experimental neurology*. 1993; 30:39–60. [PubMed: 7712628]
6. Muffley LA, Pan SC, Smith AN, Ga M, Hocking AM, Gibran NS. Differentiation state determines neural effects on microvascular endothelial cells. *Experimental cell research*. 2012; 318(16):2085–2093. DOI: 10.1016/j.yexcr.2012.06.002 [PubMed: 22683922]
7. Ydens E, Cauwels A, Asselbergh B, Goethals S, Peeraer L, Lornet G, Almeida-Souza L, Van Ginderachter JA, Timmerman V, Janssens S. Acute injury in the peripheral nervous system triggers an alternative macrophage response. *Journal of neuroinflammation*. 2012; 9:176.doi: 10.1186/1742-2094-9-176 [PubMed: 22818207]
8. Liu M, Warn JD, Fan Q, Smith PG. Relationships between nerves and myofibroblasts during cutaneous wound healing in the developing rat. *Cell and tissue research*. 1999; 297(3):423–433. [PubMed: 10460489]
9. Cruise BA, Xu P, Hall AK. Wounds increase activin in skin and a vasoactive neuropeptide in sensory ganglia. *Developmental biology*. 2004; 271(1):1–10. DOI: 10.1016/j.ydbio.2004.04.003 [PubMed: 15196945]
10. Wallengren J. Vasoactive peptides in the skin. *The journal of investigative dermatology Symposium proceedings*. 1997; 2(1):49–55. [PubMed: 9487016]
11. Rosa AC, Fantozzi R. The role of histamine in neurogenic inflammation. *British journal of pharmacology*. 2013; 170(1):38–45. DOI: 10.1111/bph.12266 [PubMed: 23734637]

12. Maggi CA. The effects of tachykinins on inflammatory and immune cells. *Regulatory peptides*. 1997; 70(2–3):75–90. [PubMed: 9272619]
13. Smith PG, Liu M. Impaired cutaneous wound healing after sensory denervation in developing rats: effects on cell proliferation and apoptosis. *Cell and tissue research*. 2002; 307(3):281–291. DOI: 10.1007/s00441-001-0477-8 [PubMed: 11904764]
14. Fukai T, Takeda A, Uchinuma E. Wound healing in denervated rat skin. *Wound Repair Regen*. 2005; 13(2):175–180. DOI: 10.1111/j.1067-1927.2005.130208.x [PubMed: 15828942]
15. Kim LR, Whelpdale K, Zurowski M, Pomeranz B. Sympathetic denervation impairs epidermal healing in cutaneous wounds. *Wound Repair Regen*. 1998; 6(3):194–201. [PubMed: 9776863]
16. Chien S. Ischemic rabbit ear model created by minimally invasive surgery. *Wound Repair Regen*. 2007; 15(6):928–935. DOI: 10.1111/j.1524-475X.2007.00285.x [PubMed: 18028143]
17. Wang J, Wan R, Mo Y, Zhang Q, Sherwood LC, Chien S. Creating a long-term diabetic rabbit model. *Experimental diabetes research*. 2010; 2010:289614.doi: 10.1155/2010/289614 [PubMed: 21234414]
18. Pradhan Nabzdyk L, Kuchibhotla S, Guthrie P, Chun M, Auster ME, Nabzdyk C, Deso S, Andersen N, Gnardellis C, LoGerfo FW, Veves A. Expression of neuropeptides and cytokines in a rabbit model of diabetic neuroischemic wound healing. *Journal of vascular surgery*. 2013; 58(3):766–775 e712. DOI: 10.1016/j.jvs.2012.11.095 [PubMed: 23755976]
19. Buckley G, Wong J, Metcalfe AD, Ferguson MW. Denervation affects regenerative responses in MRL/MpJ and repair in C57BL/6 ear wounds. *Journal of anatomy*. 2012; 220(1):3–12. DOI: 10.1111/j.1469-7580.2011.01452.x [PubMed: 22066944]
20. Davidson JM. Animal models for wound repair. *Archives of dermatological research*. 1998; 290(Suppl):S1–11. [PubMed: 9710378]
21. Paxinos, G. *The Rat Nervous System*. 4th. Academic Press; 2014.
22. Watson, C., Paxinos, G., Puelles, L. *The mouse nervous system*. London: Academic; 2012.
23. Nieuwenhuys, R., Donkelaar, HJT., Nicholson, C. *The Central Nervous System of Vertebrates*. Vol. 1. Springer — Verlag Berlin Heidelberg; 1998.
24. Tian H, Lu Y, Shah SP, Hong S. 14S,21R-Dihydroxydocosahexaenoic Acid Remedies Impaired Healing and Mesenchymal Stem Cell Functions in Diabetic Wounds. *The Journal of biological chemistry*. 2011; 286(6):4443–4453. [PubMed: 21112969]
25. Tian H, Lu Y, Shah SP, Hong S. Autacoid 14S,21R-dihydroxy-docosahexaenoic acid counteracts diabetic impairment of macrophage prohealing functions. *The American journal of pathology*. 2011; 179(4):1780–1791. [PubMed: 21839062]
26. Hong S, Alapure BV, Lu Y, Tian H, Wang Q. 12/15-Lipoxygenase deficiency reduces densities of mesenchymal stem cells in the dermis of wounded and unwounded skin. *The British journal of dermatology*. 2014; 171(1):30–38. [PubMed: 24593251]
27. Hong S, Alapure BV, Lu Y, Tian H, Wang Q. Immunohistological localization of endogenous unlabeled stem cells in wounded skin. *J Histochem Cytochem*. 2014; 62(4):276–285. [PubMed: 24399040]
28. Hong S, Lu Y, Tian H, Alapure BV, Wang Q, Bunnell BA, Laborde JM. Maresin-like lipid mediators are produced by leukocytes and platelets and rescue reparative function of diabetes-impaired macrophages. *Chemistry & biology*. 2014; 21(10):1318–1329. DOI: 10.1016/j.chembiol.2014.06.010 [PubMed: 25200603]
29. Hong S, Tian H, Lu Y, Laborde JM, Muhale FA, Wang Q, Alapure BV, Serhan CN, Bazan NG. Neuroprotectin/protectin D1: endogenous biosynthesis and actions on diabetic macrophages in promoting wound healing and innervation impaired by diabetes. *Am J Physiol Cell Physiol*. 2014; 307(11):C1058–1067. [PubMed: 25273880]
30. Lu Y, Tian H, Hong S. Novel 14,21-dihydroxy-docosahexaenoic acids: structures, formation pathways, and enhancement of wound healing. *Journal of lipid research*. 2010; 51(5):923–932. [PubMed: 19965612]
31. Richards AM, Floyd DC, Terenghi G, McGrouther DA. Cellular changes in denervated tissue during wound healing in a rat model. *The British journal of dermatology*. 1999; 140(6):1093–1099. [PubMed: 10354076]

32. Chiang HY, Chen CT, Chien HF, Hsieh ST. Skin denervation, neuropathology, and neuropathic pain in a laser-induced focal neuropathy. *Neurobiology of disease*. 2005; 18(1):40–53. DOI: 10.1016/j.nbd.2004.09.006 [PubMed: 15649695]

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Fig. 1. Microsurgical denervation of select nerves impeded the closure of full-thickness skin excision wounds of mouse ear pinnae. **a.** Representative wound photos of denervated and non-denervated ear pinnae at 0, 6, 14, and 21 days post-wounding (dpw). Alongside the wound was a thin ruler with a 1 mm divisions for reference. Arrows indicate the wounds. **b.** Wound closure was calculated as the closed area percentage relative to original wound area (at 0 dpw). The wound areas were measured from the digital wound photos using ImageJ

software. Data are mean $\pm$ SD (n = 4 mice). ** $p < 0.01$, versus denervated at the same dpw.
$p < 0.01$, versus 0 dpw.

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Fig. 2.

Microsurgical denervation of select nerves delayed the closure of full-thickness skin excision wounds of rat ear pinnae. **a.** Representative wound photos of denervated and non-denervated ear pinnae at 0, 7, and 12 days post-wounding (dpw).. **b.** Wound closure (%) measured as in Fig. 1. Data are mean $\pm$ SD (n = 4 rats). ** $p < 0.01$, versus denervated at the same dpw. ## $p < 0.01$, versus wound closure at 0 dpw.

Fig. 3. Surgical denervation of central, caudal, and rostral nerves impaired the closure of full-thickness skin excision wounds of rabbit pinnae. **a.** Representative wound photos of denervated and non-denervated ear pinnae at 0, 7, 14, and 18 days post-wounding (dpw). **b.** Wound closure (%) was measured as in Figure 2. The surgery procedures were the same as those described in supplemental Figure 1. Data are mean $\pm$ SD ($n = 4$ rabbits). ** $p < 0.01$, versus denervated wounds at the same dpw. ## $p < 0.01$, versus 0% wound closure at 0 dpw.

Fig. 4.

Surgical denervation of central, caudal, and rostral nerves reduced the re-epithelialization and granulation tissue formation of full-thickness skin excision wounds of rat or rabbit pinnae. **a.** Typical micro-images of the HE stained cryosections of excisional wounds of denervated and non-denervated ear pinnae. ep: epidermis. g: granulation tissues (circled by dashed lines). ca: cartilage. scale bar: 200 μ m. insert: an image at high magnification of the same section (scale bar: 50 μ m). Double red arrows mark the epithelial gap. **b.** Re-epithelialization, % (left) granulation tissue areas (right) for rats at 12 dpw. **c.** Re-

epithelization, % (left) and granulation tissue areas (right) for rabbits at 7 and 18 dpw. Re-epithelialization % was calculated from the epithelial gap and wound diameter at 0 dpw. Epithelial gap and granulation tissue areas were analyzed using Adobe Photoshop 9.0 software 0. Data are mean $\pm$ SD (n = 4 animals). * $p < 0.05$, ** $p < 0.01$, versus non-denervated control at the same dpw.

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