Recovery of sympathetic nerve function after lumbar sympathectomy is slower in the hind limbs than in the torso

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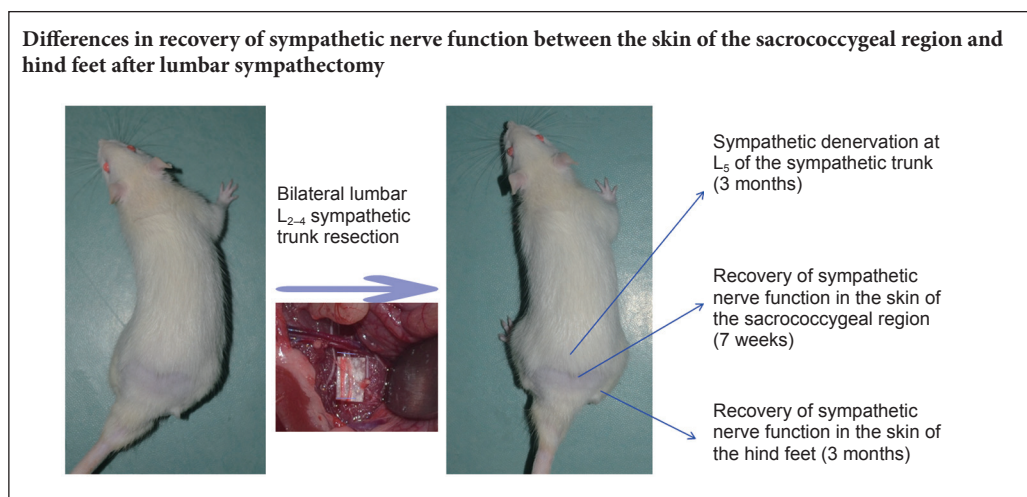5 Center of Wound Treatment, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou, Guangdong Province, China
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Graphical Abstract

Differences in recovery of sympathetic nerve function between the skin of the sacrococcygeal region and hind feet after lumbar sympathectomy

Bilateral lumbar L_{2-4} sympathetic trunk resection

Sympathetic denervation at L_5 of the sympathetic trunk (3 months)

Recovery of sympathetic nerve function in the skin of the sacrococcygeal region (7 weeks)

Recovery of sympathetic nerve function in the skin of the hind feet (3 months)

Abstract

Local sympathetic denervation by surgical sympathectomy is used in the treatment of lower limb ulcers and ischemia, but the restoration of cutaneous sympathetic nerve functions is less clear. This study aims to explore the recovery of cutaneous sympathetic functions after bilateral L_{2-4} sympathectomy. The skin temperature of the left feet, using a point monitoring thermometer, increased intraoperatively after sympathectomy. The cytoplasm of sympathetic neurons contained tyrosine hydroxylase and dopamine β-hydroxylase, visualized by immunofluorescence, indicated the accuracy of sympathectomy. Iodine starch test results suggested that the sweating function of the hind feet plantar skin decreased 2 and 7 weeks after lumbar sympathectomy but had recovered by 3 months. Immunofluorescence and western blot assay results revealed that norepinephrine and dopamine β-hydroxylase expression in the skin from the sacrococcygeal region and hind feet decreased in the sympathectomized group at 2 weeks. Transmission electron microscopy results showed that perinuclear space and axon demyelination in sympathetic cells in the L_5 sympathetic trunks were found in the sympathectomized group 3 months after sympathectomy. Although sympathetic denervation occurred in the sacrococcygeal region and hind feet skin 2 weeks after lumbar sympathectomy, the skin functions recovered gradually over 7 weeks to 3 months. In conclusion, sympathetic functional recovery may account for the recurrence of hyperhidrosis after sympathectomy and the normalization of sympathetic nerve trunks after incomplete injury. The recovery of sympathetic nerve function was slower in the limbs than in the torso after bilateral L_{2-4} sympathectomy.

Key Words: nerve regeneration; lumbar sympathectomy; sympathetic nerve; skin; recovery of function; neural regeneration

Introduction

Sympathetic denervation is associated with many diseases. Sympathetic neuropathy is frequently observed in patients with diabetes, and reflects the severity of diabetes (Faerman et al., 1982). Patients with a spinal cord injury or Parkinson's disease may also show symptoms of sympathetic denervation. Many localized lesions, such as diabetic feet (Faerman et al., 1982), limb ischemia (Stumpflen et al., 2000), Raynaud's disease (Thune et al., 2006), and hyperhidrosis (Ng and Yeo, 2003), are also strongly associated with sympathetic

nervous system function. Surgical sympathectomy is an effective method for treating such localized lesions. Thoracic sympathectomy is useful to treat digital ischemia or ulcers (Han et al., 2008; Coveliers et al., 2011). Lumber sympathectomy improves wound healing in diabetic feet (Shor et al., 2004), chronic venous leg ulcers (Patman, 1982), and critical limb ischemia (Hatangdi and Boas, 1985; Rivers et al., 1986; van Dielen et al., 1998; Tomlinson, 2000). Digital periarterial sympathectomy is the first treatment option for Raynaud syndrome (Letamendia et al., 2016).

Objective and reliable evaluation methods are essential to detect sympathetic denervation (Navarro, 2016). Useful indicators of sympathetic denervation, include sweating, warm skin, increased blood flow, electrodermal activity, pain response, skin integrity, and skin wrinkling. Norepinephrine (NE), tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DβH) are commonly used as sympathetic markers that can reflect sympathetic nerve function in the skin. TH and DβH are key enzymes involved in NE synthesis (Lehtosalo et al., 1988; Mione et al., 1991).

The skin is the largest and most superficial organ of the body; its functions change after sympathetic denervation. There has been extensive research on nerve regeneration. However, it has mainly focused on the regeneration of motor and sensory nerves. The regeneration of sympathetic nerves has been confirmed in the internal organs and cardiovascular system, but seldom in skin. Navarro et al. (1997) showed sudomotor nerve regeneration in the footpad after crush or section of the sciatic nerve in mice. However, what controls the regeneration of sympathetic nerves after lumbar sympathectomy is still unclear. In this study, we used various methods to measure the recovery from sympathetic denervation in the skin of the sacrococcygeal region and hind feet of rats after lumbar sympathectomy.

Materials and Methods

Animals

Thirty-six male Sprague-Dawley rats weighing 250–300 g were purchased from the Experimental Animal Center of Southern Medical University of China (SCXK (Yue) 2016-0041). Rats were acclimatized for at least 1 week before use. The rats were maintained at 22–24°C with a 12-hour light/dark cycle and allowed free access to standard laboratory food and water. The ambient temperature was maintained at 25°C in all animal experiments. The study protocol was approved by the Ethics Committee of Guangzhou General Hospital of Guangzhou Military Command of China (approval No. 2015030901).

Rats were randomly divided into sympathectomized ($n = 12$), sham-operated ($n = 12$), and normal groups ($n = 12$).

Rat model establishment

All rats underwent bilateral lumbar L_{2-4} sympathetic trunk resection as follows: the rats were intraperitoneally anesthetized with 0.6% pentobarbital sodium (40 mg/kg; Anthony Products, Arcadia, CA, USA). After epilation and disinfection, an incision was made along the middle of the abdomen

from the xiphoid to the pubic symphysis. The lumbar sympathetic trunks were located behind the abdominal aorta and vena cava and dissociated under a surgical microscope (M300; Leica, Mannheim, Germany) from the level of the left renal artery (L_2 sympathetic ganglia) to the level of the bifurcation in the descending aorta (L_5 sympathetic ganglia) (Hweidi et al., 1985). Bilateral L_{2-4} sympathetic trunks were removed and used for the preparation of frozen sections to test for TH and DβH activities. The increased skin temperature of the left feet, taken intraoperatively, and the positive staining of TH and DβH indicated the accuracy of sympathectomy. After complete hemostasis, the incision was closed with 5-0 sutures, and all rats were transferred to a clean cage. The rats in the sham-operated group underwent the identical surgical procedures to those in the sympathectomized group, but the bilateral lumbar sympathetic trunks were not removed. The normal group did not undergo surgery after anesthesia. All rats were transferred to a clean cage after sympathectomy. No drugs were used after the operation. After fasting for 6 hours, the rats were fed normal diets.

Immunofluorescence analysis of lumbar sympathetic trunks after operation

Freshly frozen lumbar sympathetic trunk segments from the rats were sectioned (5-μm) and fixed with ice-cold acetone for 10 minutes. The sections were immersed in ethylenediaminetetraacetic acid buffer (pH 6.0) for 20 minutes at 95°C. The sections were incubated with primary goat polyclonal anti-TH antibody (1:800 dilution; Abcam, Cambridge, UK) and sheep polyclonal anti-DβH antibody (1:800 dilution; Abcam) for 12 hours at 4°C. After rinsing with Tris-buffered saline, sections were incubated for 60 minutes at room temperature with the secondary antibody anti-goat IgG H&L antibody (Alexa Fluor® 488) and anti-sheep IgG H&L antibody (Alexa Fluor® 568) (Abcam). The slides were then incubated with Hoechst 33342 as the substrate for 3 minutes to visualize positively immunostained cells. Finally, the slides were mounted with 95% glycerol and observed under a fluorescence microscope (Olympus, Tokyo, Japan).

Temperature monitoring postoperatively

The temperature of the plantar skin of the left hind feet was recorded continuously throughout the surgery using a point monitoring thermometer (Stens Biofeedback NeXus-10 MKII; Stens Corp., San Rafael, CA, USA). The temperature of the plantar skin of the left hind feet and sacrococcygeal skin was recorded again, without anesthesia, 2 and 7 weeks after sympathectomy.

Iodine starch test

Apocrine (Robertshaw, 1974) and eccrine (Sokolov et al., 1980) sweat glands are innervated by the sympathetic nervous system. The sudomotor system is also controlled by sympathetic neurons. The iodine starch reaction, which is often used to observe the appearance of sweat spots and to evaluate areas wetted by sweat, can be more sensitive than cardiovascular tests (Ryder et al., 1988). The iodine starch test was performed on the plantar skin to evaluate sweating 2,

7 weeks, and 3 months after sympathectomy on the remaining rats. The plantar skin was covered with iodine reagent (Aladdin, Shanghai; 2 g dissolved in 100 mL anhydrous ethanol). Hydrochloride adrenaline (20 μg/100 g) was injected into the thigh muscle to induce sweating. After the plantar skin was fully dried, soluble starch (50 g in 100 mL castor sesame oil; Tianjin Damao Chemical Reagent Factory, Tianjin, China) was applied. Starch reacts with the iodine reagent giving a blue-brown color. The number of blue sweat spots was determined and photographed using a digital camera (D3100; Nikon, Tokyo, Japan) 15 minutes after starch was added to the iodine reagent. The sweating in the hind feet with sympathetic denervation will be less than that in the front feet with normal sympathetic nerve function. We defined the decreased sweat spots in hind feet with sympathetic denervation as positive staining. The number of the rats with positive staining was calculated.

Sample collection

All samples were collected under anesthesia, and the rats were subsequently euthanized. The skin of the sacrococcygeal region and hind feet was removed for immunoenzyme staining and western blot assay in 4 rats per group at 2, 7 weeks, and 3 months after sympathectomy. The L_5 sympathetic trunks at the level of the bifurcation of the descending aorta were excised and quickly placed in 4% paraformaldehyde for transmission electron microscopy (H-7500; Hitachi, Tokyo, Japan).

Immunoenzyme staining

Paraffin sections (5-μm) of skin from the sacrococcygeal region and hind feet were de-paraffinized, washed three times in phosphate buffer saline for 5 minutes, and then blocked with 5% serum for 30 minutes. The slides were subsequently incubated with sheep polyclonal anti-DβH antibody (1:800 dilution; Abcam) and rabbit polyclonal anti-NE antibody (1:1,000 dilution; Lifespan, Cambridge, UK) at 4°C overnight. After rinsing three times with phosphate buffer saline, the slides were incubated with donkey anti-sheep IgG H&L antibody (1:1,000 dilution; Abcam) and donkey anti-rabbit IgG H&L antibody (1:1,000 dilution; Abcam) at 37°C for 30 minutes. The secondary antibodies were horseradish peroxidase-labeled antibodies, further developed with 3,3′-diaminobenzidine solution. After subsequent staining with hematoxylin, the sections were observed under the microscope and images were taken with the Image-Pro Plus software (version 6.0; Media Cybernetics Corp., Silver Springs, MD, USA).

Western blot assay

The tissue lysate of the skin from the sacrococcygeal region and hind feet was homogenized in radio-immunoprecipitation assay buffer (50 mM Tris HCl, pH 8, 150 mM NaCl, 1% nonidet P40 (NP-40), 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 2 mM ethylenediaminetetraacetic acid, protease inhibitor cocktail, phosphatase inhibitor cocktail, and 1 mM dithiothreitol). Protein concentration was determined using the bovine serum albumin method and the skin lysates were denatured at 95°C for 5 minutes, in sample buffer. Forty μg of total protein was resolved using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% fat-free dry milk in Tris-buffered saline containing 0.1% (v/v) Tris-buffered saline/Tween 20 for 1 hour at room temperature. The membranes were then incubated with sheep polyclonal anti-DβH antibody (1:3,000 dilution), and rabbit anti-NE antibody (1:1,500 dilution), overnight at 4°C. Subsequently, membranes were washed and incubated for 1 hour at room temperature with donkey anti-sheep antibody (1:20,000 dilution; Abcam) or donkey anti-rabbit antibody (1:20,000 dilution; Abcam). The membranes were exposed to the enhanced chemifluorescence reagent, followed by scanning on the VersaDoc (Bio-Rad Laboratories, Portugal). For normalization, the membranes were reprobed with a rabbit polyclonal anti-GAPDH antibody (1:10,000 dilution; Abcam). Band intensities were quantified using Image-Quant TL software (GE Healthcare, Piscataway, NJ, USA). Protein levels were calculated relative to the GAPDH level in the same sample.

Transmission electron microscopy of L_5 sympathetic trunks

At 3 months after sympathectomy, the sympathetic trunks were prefixed in 4% paraformaldehyde at 4°C overnight, followed by post-fixation with 1% osmium tetroxide for 2 hours. The specimens were dehydrated with gradient alcohol series and acetone, incubated in propylene oxide/epoxy resin mixture, and embedded in pure resin. 40–60 nm sections were cut, double stained with uranyl acetate and lead citrate, and observed under transmission electron microscope (H-7500; Hitachi).

Statistical analysis

Quantitative data were expressed as the mean ± SD. All statistical computations were performed using the SPSS 21.0 software (IBM, Armonk, NY, USA). Statistical comparisons were performed using one-way analysis of variance and the least significant difference test for quantitative data and chi-square test for qualitative data. The data for analysis of variance were subjected to a Kolmogorov Smirnov Test for normal distribution, which showed that the data in every group were normally distributed. $P < 0.05$ was considered statistically significant.

Results

Assessment of rat models of sympathectomy

All rats survived the surgical procedures. The anatomy of the sympathetic trunks can vary (Miao et al., 1995). It is important to be accurate when cutting the bilateral paravertebral ganglia of the sympathetic chain during lumbar sympathectomy. The lumbar sympathetic trunks are pale and located in front of the spine on the inner side of the bilateral genitofemoral nerves, but behind the abdominal aorta and vena cava (**Figure 1**), consistent with a previous report (Hweidi et al., 1985). Care must be taken to avoid damage to large blood
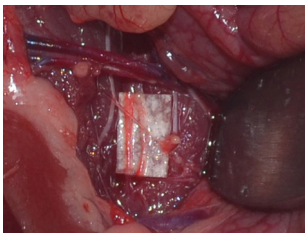
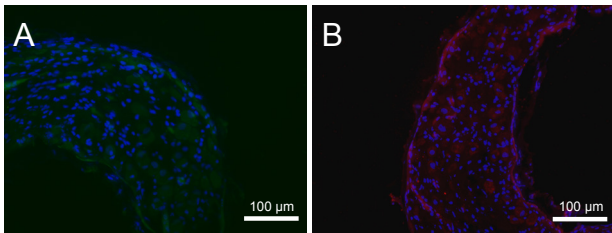**Figure 1 Anatomy of the bilateral lumbar sympathetic trunk (indicated by arrows) in a rat.**

Figure 2 Immunofluorescence staining results in the lumbar sympathetic trunk in rats under fluorescence microscope (× 200).
(A, B) Immunofluorescence staining showed positive expression of tyrosine hydroxylase (green, A) and dopamine β-hydroxylase (red, B) in the cytoplasm of sympathetic neurons in excised sympathetic trunks. Scale bars: 100 μm.
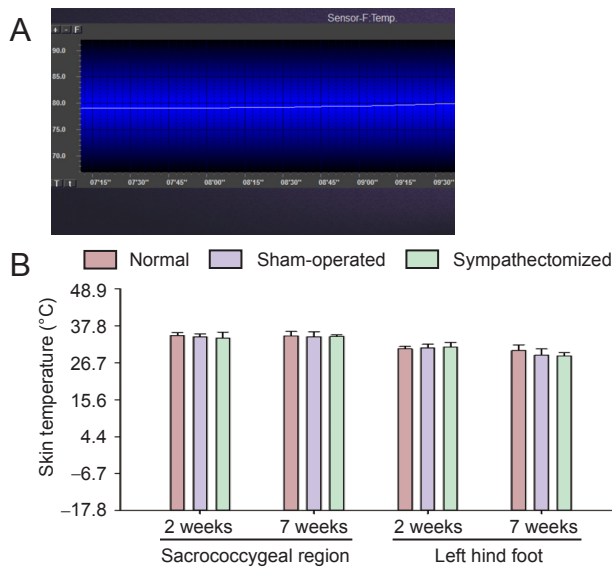

Figure 3 Skin temperature of the left feet.
(A) Intraoperative temperatures of the left feet skin during lumbar sympathectomy in rats. The abscissa represents time (seconds), and the ordinate represents temperature (°C). (B) The postoperative temperatures of the left feet skin 2 weeks ($n = 12$) and 7 weeks ($n = 8$) after sympathectomy. No significant differences in hind left plantar skin temperature or sacrococcygeal skin temperature were found among groups or at different time points ($P > 0.05$). Data are expressed as the mean ± SD (one-way analysis of variance and the least significant difference test).

Figure 4 Effect of lumbar sympathectomy on the sweating of the plantar skin of the hind feet.
Negative staining: Blue sweat spots in the plantar skin of hind feet were not different from those in the plantar skin of front feet in the rats without sympathetic denervation. Positive staining: Blue sweat spots in the plantar skin of hind feet were fewer than those in the plantar skin of front feet in the rats with lumbar sympathetic denervation.

Figure 5 Immunohistochemical staining of norepinephrine under a light microscope in the plantar skin of hind feet 2 weeks and 3 months after sympathectomy in rats.
At 2 weeks, the positive staining of norepinephrine in the sympathectomized group was less than that in the normal group. Arrows indicate positive staining within sympathetic neurons. Scale bars: 100 μm.

vessel during surgery. If the small blood vessels around the sympathetic trunks are damaged, hemostasis must be restored. It is important to note the differences between the lumbar sympathetic trunk and genitofemoral nerve. The lumbar sympathetic trunk is gray with many ganglia branches. Genitofemoral nerves are white and have no branches between spinal segments 2 and 4.
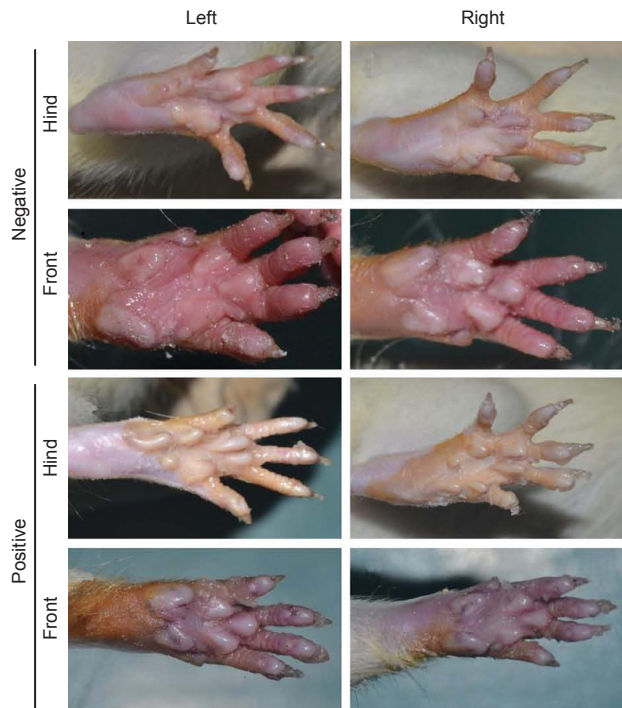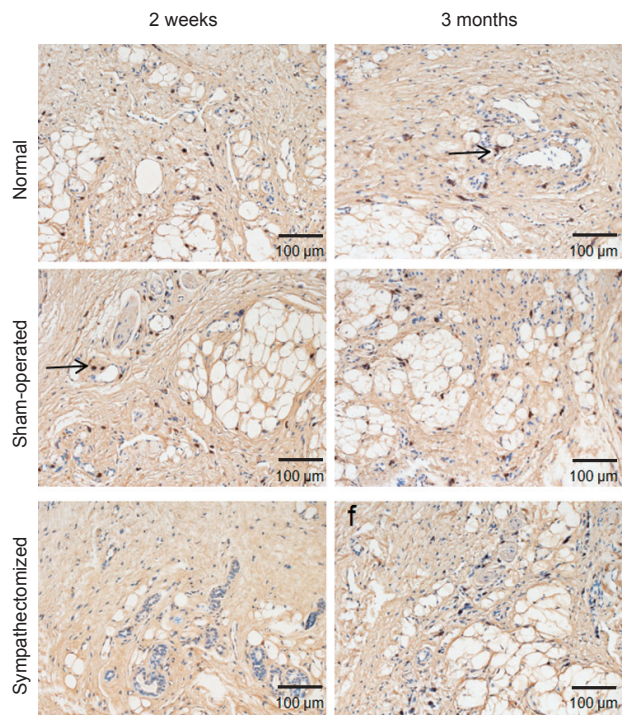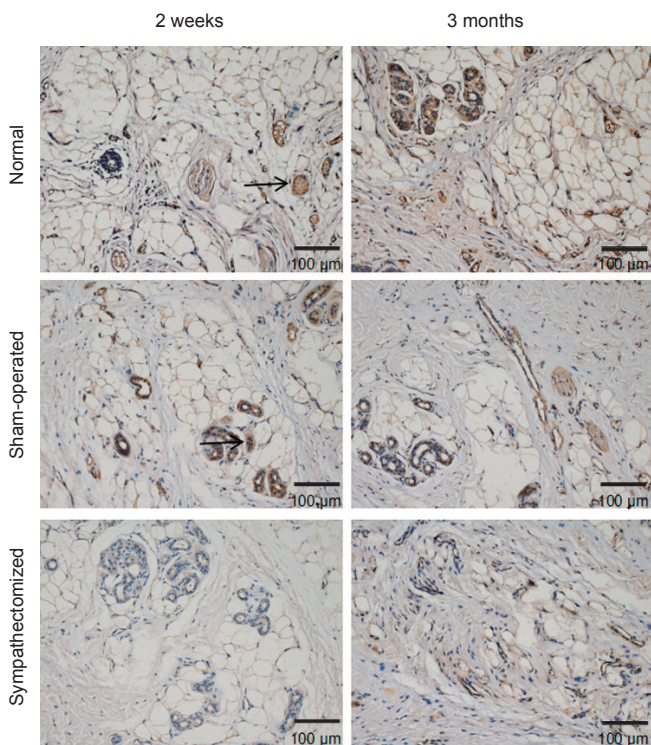
Figure 6 Immunohistochemical staining of dopamine β-hydroxylase under a light microscope in the plantar skin of hind feet 2 weeks and 3 months after sympathectomy in rats.

At 2 weeks, the positive staining of dopamine β-hydroxylase in the sympathectomized group was less than that in the normal group. Arrows indicate positive staining within sympathetic neurons. Scale bars: 100 μm.

Figure 8 Immunohistochemical staining of norepinephrine under a light microscope in sacrococcygeal skin 2 and 7 weeks after sympathectomy in rats.

At 2 weeks, the positive staining of norepinephrine in the sympathectomized group was less than that in the normal group. Arrows indicate positive staining within sympathetic neurons. Scale bars: 100 μm.
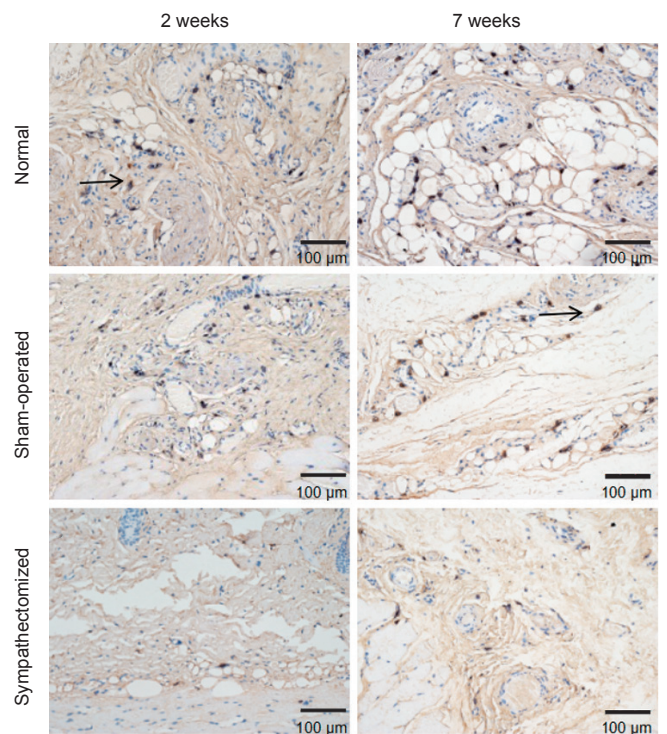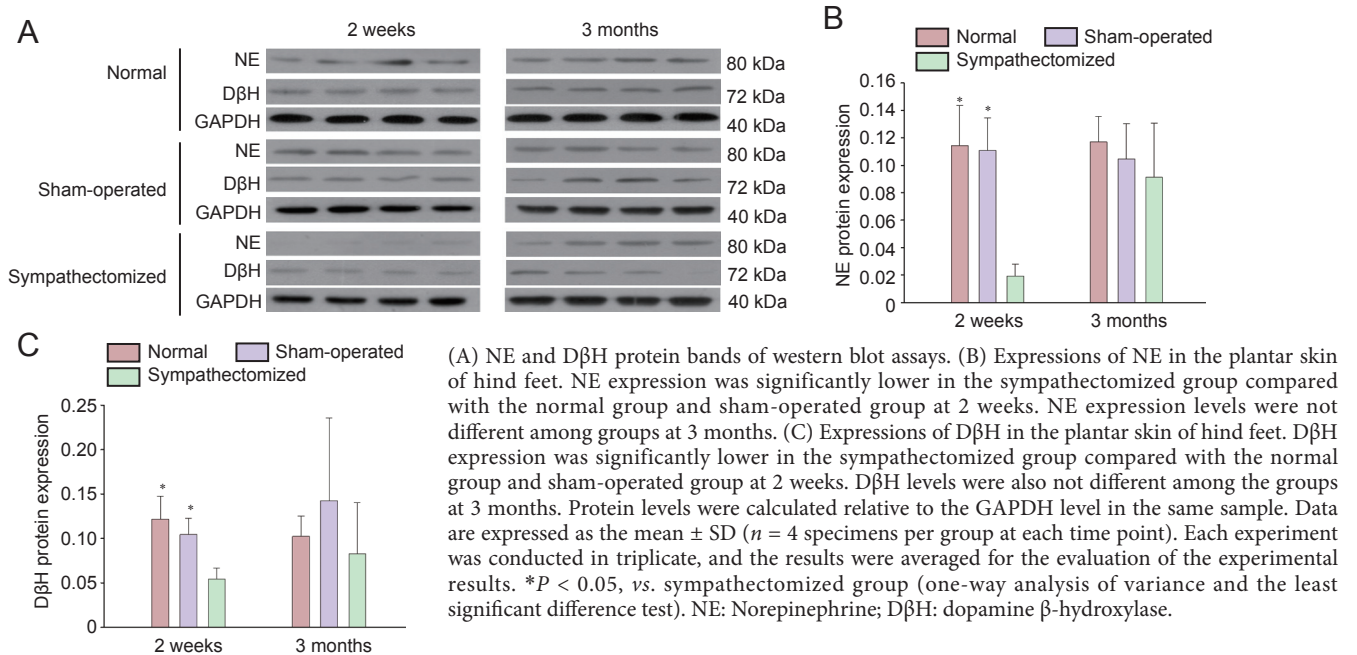

(A) NE and DβH protein bands of western blot assays. (B) Expressions of NE in the plantar skin of hind feet. NE expression was significantly lower in the sympathectomized group compared with the normal group and sham-operated group at 2 weeks. NE expression levels were not different among groups at 3 months. (C) Expressions of DβH in the plantar skin of hind feet. DβH expression was significantly lower in the sympathectomized group compared with the normal group and sham-operated group at 2 weeks. DβH levels were also not different among the groups at 3 months. Protein levels were calculated relative to the GAPDH level in the same sample. Data are expressed as the mean ± SD ($n = 4$ specimens per group at each time point). Each experiment was conducted in triplicate, and the results were averaged for the evaluation of the experimental results. *$P < 0.05$, *vs.* sympathectomized group (one-way analysis of variance and the least significant difference test). NE: Norepinephrine; DβH: dopamine β-hydroxylase.

Figure 7 Protein expression of NE and DβH in the plantar skin of hind feet 2 weeks and 3 months after sympathectomy in rats.

Expression of TH and DβH in the cytoplasm of sympathetic neurons in excised sympathetic trunks

The cytoplasm of sympathetic neurons in excised sympathetic trunks was positive for TH (**Figure 2A**) and DβH (**Figure 2B**), indicating the efficacy of the sympathectomy. The cell bodies and nuclei in the sympathetic neurons were larger than those in other cells in the lumbar sympathetic trunks.

Skin temperature

The skin temperature of the left feet increased by more than 0.8°C intraoperatively, 30 minutes after the lumbar sympathetic trunk resection (**Figure 3A**), which is considered an indication of successful sympathetic resection (Greenstein et al., 1994). No significant differences were found in the sacrococcygeal skin temperature at 2 weeks ($P > 0.05$) or at 7 weeks ($P > 0.05$) among the normal group, sham-operated group and sympathectomized group. Similarly, no significant differences were found in the plantar skin of the left hind feet among the groups 2 and 7 weeks after sympathectomy or between different time points ($P > 0.05$) (**Figure 3B**).

Effect of lumbar sympathectomy on sweating by the plantar skin of the hind feet

Fewer blue sweat spots were detected on the plantar skin of the hind feet than on the plantar skin of the front feet in rats with sympathetic denervation (positive staining; **Figure 4**). In contrast, blue sweat spots in the plantar skin of hind feet were not different from those in the plantar skin of the front feet in rats without sympathetic denervation (negative staining; **Figure 4**). Positive staining was seen in 12/12, 3/12, and 1/12 rats at 2, 7 weeks and 3 months respectively in the sympathectomized group. No rats in the control groups stained positive with the iodine starch test. The difference in the number of the rats with positive staining between the sympathectomized and other groups was significant at 2 and 7 weeks ($P < 0.001$) but not at 3 months ($P > 0.05$).

NE and DβH expression in the plantar and sacrococcygeal skin of the hind feet

NE and DβH were mainly localized in arteriovenous anastomoses, arrector pilorum muscles and arterioles, whereas few adrenergic fibers were found around the sweat glands (**Figures 5, 6**); (Donadio et al., 2006). At 2 weeks post-surgery, NE and DβH expression in the plantar skin of the hind feet was significantly lower in the sympathectomized group compared with the normal group (both $P < 0.05$). NE ($P < 0.001$ and DβH ($P = 0.006$) expression in the sympathectomized group significantly decreased compared with the sham-operated group at 2 weeks in the plantar skin of the hind feet. NE expression levels were not different among the normal, sham-operated and sympathectomized groups at 3 months in the plantar skin of the hind feet ($P > 0.05$). DβH levels were also not different among the groups at 3 months in the plantar skin of the hind feet ($P > 0.05$; **Figure 7**).

NE and DβH levels in the sacrococcygeal skin are shown in **Figures 8–10**. NE expression had decreased significantly at 2 weeks in the sympathectomized group compared with the normal and sham-operated groups (both $P < 0.05$). DβH expression was also significantly lower in the sympathectomized group than in the normal and sham-operated groups in the sacrococcygeal skin at 2 weeks (both $P < 0.05$). At 7 weeks, NE expression levels in the sacrococcygeal skin were not different among the normal, sham-operated and sympathectomized groups ($P > 0.05$). Similarly, there was no significant difference in DβH expression levels in the sacrococcygeal skin among the normal, sham-operated and sympathectomized groups at 7 weeks ($P > 0.05$; **Figure 10**).

Ultrastructure of the L₅ sympathetic trunks

The L_5 sympathetic trunks in the sympathectomized and sham-operated groups were observed under transmission electron microscope at 3 months after sympathectomy (**Figure 11**). Regeneration of the L_{2-4} sympathetic trunks did not occur, and intestinal adhesions were found. The ultrastructure showed integral myelin in the axons and no perinuclear spaces in the sympathetic cells of the sham-operated group (**Figure 11A**). However, perinuclear space in sympathetic cells and axon demyelination were found in the sympathectomized group (**Figure 11B**). The nuclei of sympathetic neurons in the sympathectomized group were irregular, whereas those in the normal group were round. More lysosomes and rough endoplasmic reticulum were present in the sympathectomized group than in the sham-operated group.

Discussion

The sympathetic nervous system plays an important role in various skin diseases. Sympathetic denervation can be a clinical manifestation of some skin diseases, and serve as a treatment for others. Sympathetic nerve function can be restored to some extent after sympathectomy in clinical practice; severe histological damage in the sympathetic trunk almost normalizes 12 weeks after thoracoscopic clipping of the sympathetic trunk in patients with disabling primary hyperhidrosis or facial blushing (Thomsen et al., 2014). Hyperhidrosis and pain disorders may recur in patients even after treatment by thoracic sympathectomy (Johnson et al., 1999). Plantar hyperhidrosis (Rieger et al., 2011) and neuralgia (Buche et al., 1988) can recur after resection of the lumbar sympathetic trunk. Although recovery of sympathetic function in skin has been found in clinical patients, few animal models have been reported that observed and verified the regularity of this recovery.

Previous animal studies have shown sympathetic denervation in superficial pineal gland (Zhang et al., 1991; Hernandez et al., 1994) and the heart (Lindpaintner et al., 1987) after surgical sympathectomy. Major portions of the hind leg (Miao et al., 1995; Catre and Salo, 1999) and femoral arteries (Peterson and Norvell, 1985) are innervated by the L_{2-4} levels of the sympathetic chain. If the bilateral L_{2-4} sympathetic trunks are removed, the sympathetic nerve function in the lower limbs would decline. In this experiment, the lumbar sympathetic trunks were exposed clearly and cut success-

fully, as demonstrated by intraoperative monitoring of skin temperature and immunofluorescence analysis of the lumbar sympathetic trunks. Monitored skin temperature, sweating and NE and DβH proteins reflect the denervation of sympathetic nerve function in the skin 2 weeks after lumbar sympathectomy, in accordance with the previous studies (Flotte, 1959; Mashiah et al., 1995; Shor and Chumak Iu, 2001; Dellon et al., 2012). Long-term postoperative follow-up of the skin temperature after sympathectomy is rare (Hatangdi and Boas, 1985; Kruse, 1985; Greenstein et al., 1994). In our study, no significant change in skin temperature was observed between the left feet and sacrococcygeal region 2 and 7 weeks after sympathectomy. Jeong et al. (2006) also reported that skin temperature of the palms gradually begins to normalize approximately 1 week after bilateral thoracic sympathectomy.

Some reports have shown that the effects of surgical sympathectomy are time-dependent in splanchnic organs, mesenteric arteries and veins (Lehtosalo et al., 1988) and the smooth muscle of myenterically and extrinsically denervated rat jejunum (Luck et al., 1993). Baroreflex regulation of renal sympathetic nerve activity may partially recover after spinal cord hemisection in rats (Zahner et al., 2011). In this paper, the recovery of sympathetic nerve function in the hind feet and sacrococcygeal skin after lumbar sympathectomy was explored. The rate of the recovery of sympathetic nerve function in the hind feet was slower than that in the sacrococcygeal skin. The recovery of sympathetic nerve function was verified by the recovery of monitored skin temperature, sweating and expressions of NE and DβH protein in skin. To determine whether the sympathetic nerve function had completely regenerated after lumbar sympathectomy, the ultrastructure of the L_5 sympathetic trunk was examined by transmission electron microscopy. The results showed that the L_5 sympathetic trunk had not returned to its normal state 3 months after the sympathectomy.

Navarro (2009) suggested that the reinnervation of denervated segments could occur *via* regeneration of injured axons or collateral branching of undamaged axons. Sympathetic preganglionic neurons consist of cells in the lateral grey column from T_1 to $L_{2/3}$, which synapse with their postganglionic neurons, including paravertebral ganglia of the sympathetic chain, prevertebral ganglia, and chromaffin cells of the adrenal medulla. Kobayashi et al. (2001) reported that the sympathetic pathways from the canine hypogastric nerve to the seminal tract could be reconstructed spontaneously after serious injury due to their cross-innervation system. In our experiment, prevertebral ganglia and chromaffin cells of the adrenal medulla remained after resection of bilateral lumbar sympathetic trunks, and may have been involved in the compensatory recovery of sympathetic nerve function in sacrococcygeal skin and the hind feet.

The reason for the faster recovery of sympathetic nerve function in the sacrococcygeal skin is still unclear. Compared with the hind feet, the sacrococcygeal skin is closer to the adjacent skin with normal sympathetic nerve function. Postganglionic sympathetic nerves in the abdomen can regenerate and reinnervate after removal of sympathetic ganglia (Yamada et al., 2006). Therefore, it is possible that the sympathetic nerve in the sacrococcygeal skin regenerated from the adjacent normal skin after lumbar sympathectomy.

In summary, sympathetic functional recovery may account for the recurrence of hyperhidrosis after sympathectomy and normalization of sympathetic nerve trunks after an incomplete injury. The recovery of sympathetic nerve function in the sacrococcygeal skin was quicker than that in the hind feet. The slower recovery of sympathetic nerve function in the limbs than in the torso may also be the general pattern of sympathetic functional recovery in the patients after injury to sympathetic nerve trunks. We will investigate this future research.

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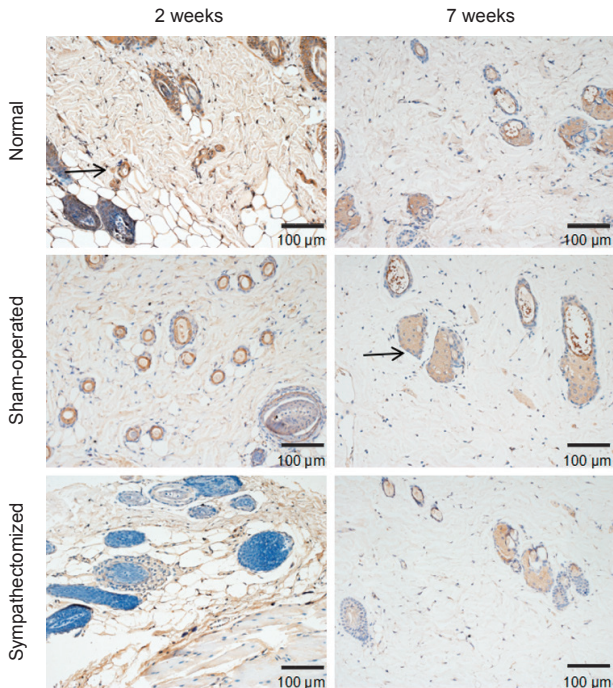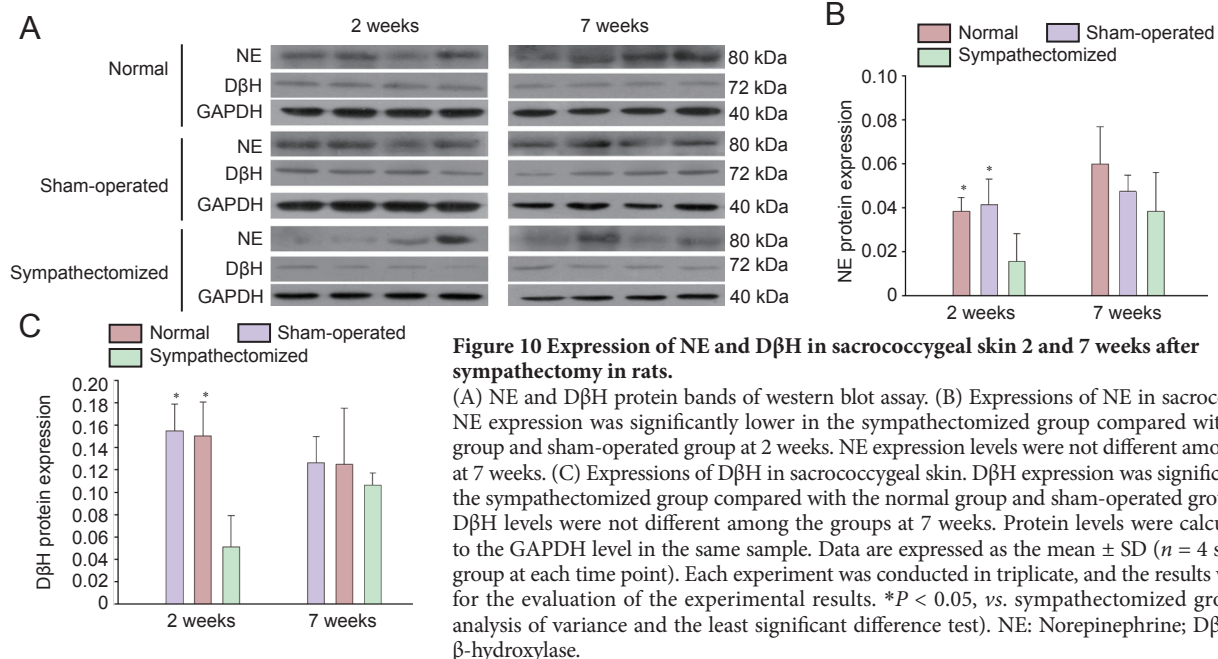

Figure 9 Immunohistochemical staining of dopamine β-hydroxylase under a light microscope in sacrococcygeal skin 2 and 7 weeks after sympathectomy in rats.

At 2 weeks, the positive staining of dopamine β-hydroxylase in the sympathectomized group was less than that in the normal group. Arrows indicate positive staining within sympathetic neurons. Scale bars: 100 μm.

Figure 10 Expression of NE and DβH in sacrococcygeal skin 2 and 7 weeks after sympathectomy in rats.

(A) NE and DβH protein bands of western blot assay. (B) Expressions of NE in sacrococcygeal skin. NE expression was significantly lower in the sympathectomized group compared with the normal group and sham-operated group at 2 weeks. NE expression levels were not different among the groups at 7 weeks. (C) Expressions of DβH in sacrococcygeal skin. DβH expression was significantly lower in the sympathectomized group compared with the normal group and sham-operated group at 2 weeks. DβH levels were not different among the groups at 7 weeks. Protein levels were calculated relative to the GAPDH level in the same sample. Data are expressed as the mean ± SD (n = 4 specimens per group at each time point). Each experiment was conducted in triplicate, and the results were averaged for the evaluation of the experimental results. *$P < 0.05$, *vs.* sympathectomized group (one-way analysis of variance and the least significant difference test). NE: Norepinephrine; DβH: dopamine β-hydroxylase.
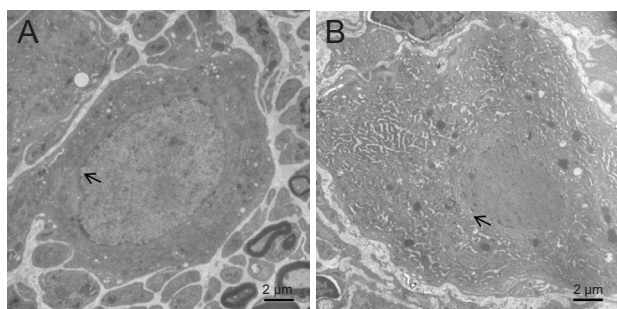
Figure 11 Effects of lumbar sympathectomy on the ultrastructure of L₅ sympathetic trunks of rats (transmission electron microscopy, uranyl acetate staining).
(A) Sympathetic cells in the sham-operated group show integral myelin of axons and no perinuclear space. (B) Demyelination of axons and perinuclear space in sympathetic cells (arrows) were found in the sympathectomized group, 3 months post-surgery. Scale bars: 2 μm.

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