COMPETITION BETWEEN DORSAL ROOT REFLEX AND SYMPATHETIC ACTIVITY ON NEUROGNEIC INFLAMMATION

by

AILING LI

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ABSTRACT

COMPETITION BETWEEN DORSAL ROOT REFLEX AND SYMPATHETIC ACTIVITY ON NEUROGENIC INFLAMMATION

Ailing Li, M.S.

The University of Texas at Arlington, 2011

Supervising Professor: Dr. Yuan Bo Peng

Neurogenic inflammation is caused by the inflammatory substances released from the peripheral terminals of afferent fibers. Vasodilation is one of the cardinal signs of neurogenic inflammation. It is thought that neurogenic inflammation is closely related with dorsal root reflex (DRR). DRRs are the antidromic action potentials that can be elicited by noxious stimuli and promote the release of inflammatory substances from the nerve terminals to facilitate neurogenic inflammation, like increase of the vasodilation. Some studies have proved that DRR can propagate bilaterally after being induced unilaterally. However, in our previous studies, we didn't observe significant increase of contralateral blood perfusion, which was supposed to be caused by contralateral propagation of DRRs, after ipsilateral chemical or electrical stimulation. In this study, I investigated whether this contralateral DRRs effect was masked by competitive sympathetic vasoconstriction. The results indicated that activation of sympathetic preganglionic fiber by stimulating L4 ventral root had vasoconstriction effect. The blood perfusion of bilateral hind paws decreased at the electrical stimulation. Blood perfusion of ipsilateral hind paw significantly increased after capsaicin injection. There was no significant increase in the blood perfusion for contralateral hind paw, in contrast, the blood perfusion tended to decrease at the moment of capsaicin

injection. There was no difference between the sympathectomized and sham groups. In conclusion, the contralateral DRR effect was not masked by sympathetic vasoconstriction. The contralateral DRR is too weak by itself to induce any changes in the blood perfusion.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

Figure Page

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Neurogenic inflammation and vasodilation

The neurogenic inflammation is the inflammation that is caused by the substances released from the afferent nerve terminals, such as substance P and CGRP. The four cardinal signs of inflammation are known as heat, redness, edema and pain (Lewis, 1927). The first two signs are caused by the vasodilation. CGRP is a potent vasodilator (Brain, Williams, Tippins, Morris, & MacIntyre, 1985b). The neurogenic vasodilation was selectively abolished by depleting endogenous CGRP or using antagonist $CGRP_{8-37}$ (Chu et al., 2001; Kawasaki, Takasaki, Saito, & Goto, 1988). There are two different mechanisms that have been proposed for the relaxant effect of CGRP, the endothelium-dependent and endothelium-independent relaxation. Brain, et al. (1985a) discovered that CGRP combined with CGRP1 receptor and had a relaxant effect on artery preparation in vitro by an endothelial cell-dependent mechanism. Grace, Dusting, Kemp, and Martin(1987) and Gray and Marshall (1992a) found that CGRP relaxation depended on intact endothelium. While Greenberg, Rhoden, and Barnes (1987) and Edvinsson, Fredholm, Hamel, Jansen, and Verrecchia (1985b) discovered that CGRP-induced relaxation was not affected by removal of endothelial cells. There are also some mediators involved in CGRP-induced vasodilation. CGRP-induced relaxation is closely related with the cAMP level (Edvinsson, Fredholm, Hamel, Jansen, & Verrecchia, 1985a; Kubota et al., 1985). CGRP combines with $CGRP_1$ receptor which is cAMP-coupled G protein to increase cAMP level. Enhanced cAMP could induce decrease of $[Ca^{2+}]_i$ through inhibition of Ca^{2+} influx due to a hyperpolarization by the stimulation of Ca^{2+}-activated K^+ channels, increased Ca^{2+} uptake into the intracellular stores and an increase of Ca^{2+} extrusion from cells through sarcolemmal Ca^{2+}-pump (Nishimura J, 2006). The decreased $[Ca^{2+}]_i$ thus causes vasodilation, since calcium is the major stimulus for muscle contraction. Inhibiton of nitric oxide synthesis by nitric oxide synthase inhibitor N^G-Monomethyl-L-arginine, N^G-nitro-L-arginine, or N^{ω}-nitro-L-arginine could inhibit CGRP-induced

relaxation (Takahashi, De Vroomen, Roman, & Heymann, 2000b; Abdelrahman, Wang, Chang, & Pang, 1992; Gray & Marshall, 1992b). It is controversial whether K_{ATP} channel is involved in CGRP-induced vasodilation (Takahashi, De Vroomen, Roman, & Heymann, 2000a; Abdelrahman et al., 1992).

SP is one of the major transmitters that are responsible to plasma extravasation (Holzer, 1998c). SP influences the permeability through major two ways: first, SP can increase vascular permeability by direct action on NK_1 receptors on the endothelium of postcapillary venules; second, SP can induce secondary mediators released from mast cells (Holzer, 1992; Holzer, 1998b; Brain & Cox, 2006). The direct action on NK_1 receptors results in the early phase of edema, while the mediators from mast cells cause the second phase of edema (Holzer, 1992). There are some other studies pointed out that there was an interaction between SP and CGRP. SP can regulate the vasodilator activity of CGRP, because co-injection of CGRP with SP into human skin converts the long lasting vasodilatation induced by CGRP into a transient response by releasing of proteases from mast cells stimulated by SP (Brain & Williams, 1988). CGRP was able to enhance exudative responses to sensory nerve stimulation, tachykinins, and a variety of inflammatory mediators (Brain & Cambridge, 1996). Further evidence showed that SP is mainly released from C-fiber, while CGRP is produced by Aδ-fiber (Janig & Lisney, 1989). Activation of Aδ-fiber could cause vasodilation, not plasma extravasation; and the magnitude and time course of the vasodilation depended on the number and frequency of stimuli delivered to the nerve. Detectable blood flow change appeared when stimulating intensity was 0.5v, and a train of 100 or so stimuli delivered at 5-10 Hz could cause greatest response (Janig et al., 1989).

Other peptides have been reported to influence the vasodilation and plasma extravasation. Neurokinin A which is one member of tachykinin family can mediate venular permeability (Holzer, 1998d); vasoactive polypeptide (VIP) and the related pituitary adenylate cyclase-activating peptide (PACAP) also play a role in neurogenic vasodilatation. Neuropeptides released after afferent nerve stimulation can also recruit and induce leukocytes to release leukocyte-derived mediators which are vasoactive and are likely to exert a modulatory action on vessel diameter and vascular permeability (Holzer, 1998a).

1.2 DRR and vasodilation

Dorsal root reflex (DRR) is the antidromic propagation of action potential. Action potential not only propagates orthodromically from periphery to central nervous system, but also propagates antidromically from spinal cord to periphery (Fig 1.1). Since Gotch and Horsley (1891) first described the phenomenon of antidromic action potential, DRR has been studied extensively. It was reviewed by Willis (1999a) that the action potentials from peripheral nerve arriving spinal cord cause the primary afferent depolarization (PAD). PAD is thought to be resulted from the GABA released from spinal cord interneuron. GABA can bind with the $GABA_A$ receptors located on the presynaptic terminals of primary afferent nerve, whereby the chloride channels open and cause the efflux of chloride ions because of the intracellular higher concentration gradient of chloride ions maintained by NKCC1 (sodium-potassium-chloride co-transporter) cross the presynaptic terminal membrane (Price, Hargreaves, & Cervero, 2006; Price, Cervero, Gold, Hammond, & Prescott, 2009; Alvarez-Leefmans et al., 2001; Alvarez-Leefmans, Gamio, Giraldez, & Noguern, 1988). This could generate the PAD. When the PAD is big enough, it will surpass the threshold to produce action potential which is the antidromic action potential DRR (Fig 1.1).

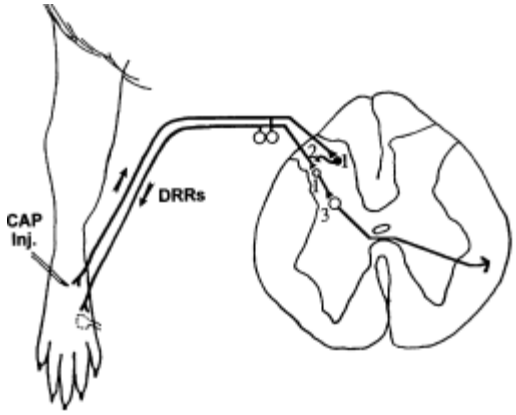


Figure 1.1 Diagram of dorsal root reflex (Adopted and modified from (Willis, 1999b). Stimulation (capsaicin injection) activates peripheral terminals of afferents. Action potentials are transmitted orthodromically to spinal cord and activate dorsal horn circuits that results in dorsal root reflexes (DRRs) propagating antidromically which produce vasodilation in the adjacent skin.

DRR was found to play a very important role in peripheral neurogenic inflammation (Lin, Wu, & Willis, 1999a). Antagonists of $GABA_A$, non-NMDA or NMDA receptors can reduce the capsaicin-induced increase in DRR, as well as the capsaicin-evoked increase in blood flow at distal site. Sections of sciatic and femoral nerve or dorsal rhizotomy could also nearly completely abolish the capsaicin-evoked increase in blood flow at distal site. But in our lab, after the application of lidocaine on the sciatic nerve, the increase of blood flow after formalin injection was significantly higher than the group without lidocaine treatment (Hagains, Trevino, He, Liu, & Peng, 2010b), which was probably due to the elimination of sympathetic component in sciatic nerve. The blood flow was not decreased after the lidocaine application on the sciatic nerve which suggests that axonal reflex play more important role than DRR in neurogenic inflammation. The reason for the discrepancy between these two studies is not clear, however, the blood flow was recorded from points in Lin's study, while blood flow was recorded from whole paw in our lab. Mimicking DRR by stimulating the distal end of dorsal root can produce vasodilation and plasma extravasation, which are cardinal signs of neurogenic inflammation (Lin, Wu, & Willis, 1999b; Chahl, 1988; Kolston & Lisney, 1993; Szolcsanyi J, 1988; Gee, Lynn, & Cotsell, 1997). DRR can activate the primary afferent terminals to release substance P (SP) and calcitonin gene-related peptide (CGRP) to produce the vasodilation and plasma extravasation. Early, it is found that smaller and medium sized afferents provide the main paths for DRR, because only when cold block was intense enough to stop propagation at speeds characteristic of smaller A fibers, DRR disappeared completely (Brooks CM & Koizumi K, 1956). Later, Lin, Zou and William (2000) found that DRR can be recorded from both small myelinated (A_δ) and unmyelinated (C) afferent fibers, as well as large mylinated (A_β) fibers. Even though, only DRRs of A_δ and C fibers significantly increased in their activities after capsaicin injection, it suggested that the centrally mediated antidromic activity in A_δ and C primary afferent fibers are the ones that contributes to the development of neurogenic inflammation.

1.3 Sympathetic activity and blood perfusion

The sympathetic system is one of the autonomic nervous systems which include sympathetic, parasympathetic and enteric systems. The sympathetic system innervates smooth muscle, cardiac muscle,

and glandular tissues and mediates a variety of visceral reflexes (Iversen, Iversen, & Saper, 2000). The motor component of sympathetic system is composed of preganglionic neurons and postganglionic neurons. Preganglionic neurons form a column in the intermediolateral horn of the spinal cord extending from the first thoracic spinal segment to rostral lumbar segments (Fig 1.2). The axons of preganglionic neurons leave the spinal cord in the ventral root and initially run together with somatic motor axons in the spinal nerve. The preganglionic axons then separate from the somatic motor axons and project through the white myelinated rami to the ganglia of the sympathetic chains, which lie along each side of the spinal cord. Axons of preganglionic neurons exit the spinal cord at the level at which their cell bodies are located, but they may also innervate sympathetic ganglia situated at other levels. The axons of postganglionic neurons are largely unmyelinated and exit the ganglia through gray unmyelinated rami. The postganglionic cells that innervate the head are located in the superior cervical ganglion, and other postganglionic fibers innervating the rest of the body travel in spinal nerves to their targets, like blood vessels, internal organs, sweat glands, etc. Preganglionic fibers primarily use acetylcholine and norepinephrine as transmitters, while most postganglionic sympathetic neurons use norepinephrine as transmitter acting on different adrenergic receptors. Norepinephrine stimulates alpha receptors which are present in the muscle within the walls of blood vessels of extremities to contract muscle, thus narrow the blood vessels. (Iversen et al., 2000)

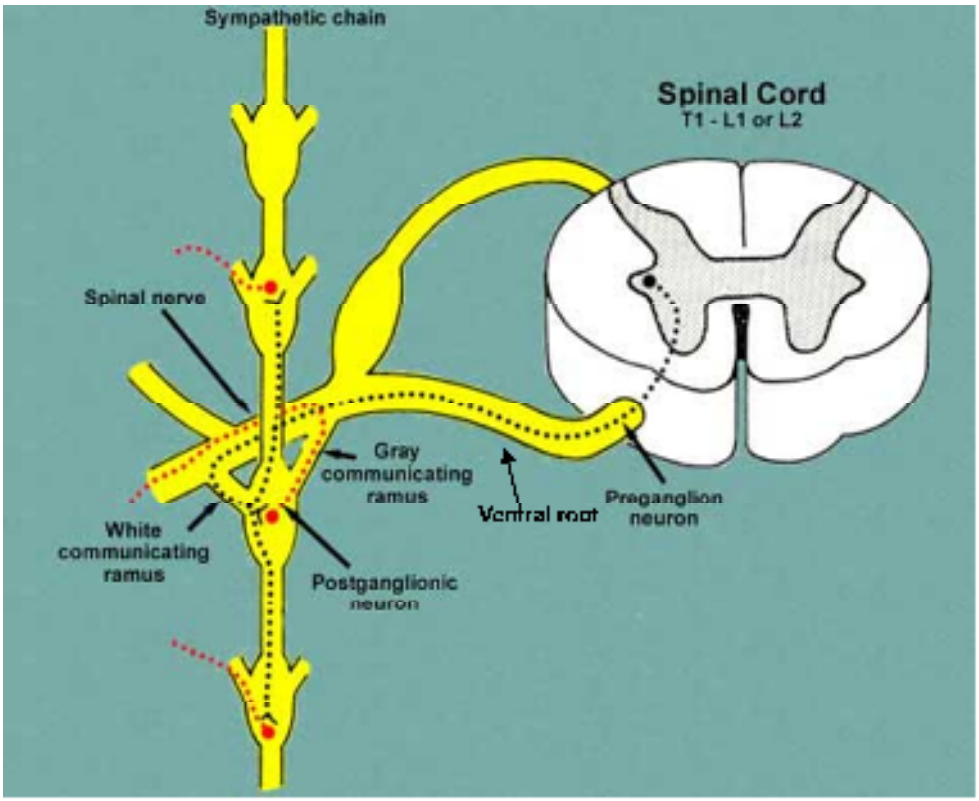


Figure 1.2 Schematic illustration of sympathetic flow (Adopted from http://home.comcast.net/~wnor/thoraxlesson5.htm)

So, the blood vessel not only can be regulated by DRR, but also controlled by activity of sympathetic nervous system. Sympathetic nervous system has long been known of its capability of blood vessel constriction, which can decrease the blood flow, increase the blood vessel resistance. Stimulation of T11-L1 ventral root could decrease nerve blood flow of sciatic nerve (Sato, Sato, & Uchida, 1994). There is a competition between sympathetic activity and DRR on the blood flow control. When sympathetic stimulation frequency >= 5 Hz by stimulating the lumbar sympathetic trunk after sympathetic trunk was cut caudally to ganglion L2 or L3, antidromic vasodilation produced by stimulating L5 dorsal root was reduced. And total suppression of neurogenic vasodilation was seen during a sympathetic stimulation with 20 Hz (Habler, Wasner, & Janig, 1997).

1.4 Contralateral effect

There are a lot of examples that unilateral intervention produce bilateral effects. Those contralateral effects have often been incidental control observations. Following peripheral-nerve lesions, like sciatic nerve, muscle nerve and hypoglossal nerve, contralateral effects were found, such as changes of RNA level, peptide levels, receptors levels, et al. (Koltzenburg, Wall, & McMahon, 1999a). One possible mechanism used to explain the contralateral effect is that commissural interneurons might have the capacity to mediate specific contralateral changes. There is transmedian signaling from ipsilateral side to contralateral side via interneurons (Fig 1.3) (Koltzenburg, Wall, & McMahon, 1999b). Actually, there were several early studies that had proved the projection from one dorsal horn to another. Fitzgerald (1982) found that 54% cells in laminaer 4, 5 and 6 have inhibitory contralateral fields, and in most cases the inhibitory field was mirror image of the excitatory ipsilateral field, which suggested a local network involving connections at one level of the cord. Later, Fitzgerald (1983) found that stimulation came from the contralateral side would excite the superficial dorsal horn neurons, substantia nigra, while the same stimulation would exert more inhibition to deeper dorsal horn laminae 4, 5 and 6.

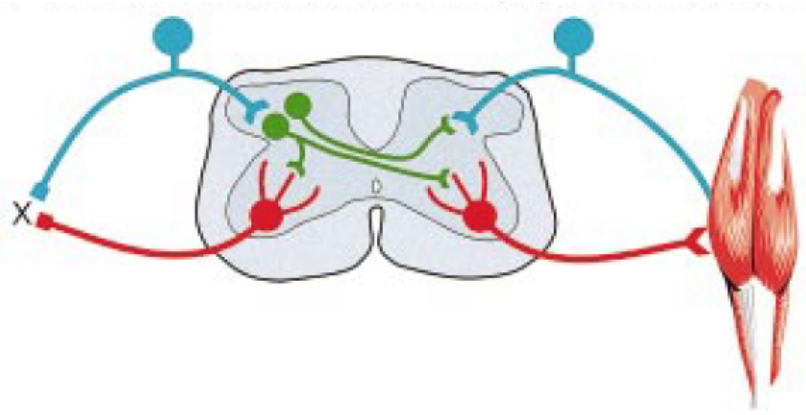


Figure 1.3 Putative mechanism by which contralateral change might occur (Adopted from Koltzenburg et al., 1999).

Based on this local spinal circuit, we would expect that DRR will not only propagate ipsilaterally, but also cross over to the other side. Indeed, there are some evidences that proved the bilateral conduction of DRRs. Bagust, Kerkut and Rakkah (1989) discovered the weaker response with longer latency in the

contralateral dorsal root compared with ipsilateral dorsal roots. Later, Bagust, Chen and Kerkut (1993) again proved that the spread of DRRs both ipsilaterally and contralaterally to the stimulated afferent input using isolated hamster spinal cord. Both the stimulations of ipsilateral and contralateral peripheral nerves could produce DRRs (Bo Peng, Kenshalo, & Gracely, 2003).

1.5 Experimental hypotheses and objectives

Since DRR can travel bilaterally, consequently we would expect bilateral vasodilation after unilateral noxious stimulation. However, based on previous studies in our lab, there was only slightly graduate increase in contralateral blood flow after ipsilateral noxious stimulation, as opposed to the immediately large increase on the ipsilateral side (Hagains, Trevino, He, Liu, & Peng, 2010a). This study aims to investigate whether the weak or non-significant contralateral increase of blood flow after capsaicin injection due to the cancellation of DRR by sympathetic activity. Capsaicin is capable of inducing acute neurogenic inflammation.

1.5.1 Specific aim 1: Determine and verify the effect of direct stimulation of ventral root L4 on the blood flow of hind paw

The ventral root includes both motor nerve and sympathetic nerve; we would expect that the stimulation of ventral root can directly activate the sympathetic activity. In addition, ventral root L4 innervates hind paw area. So, for this specific aim, It is hypohtesized that stimulation of ventral root L4 will reduce the cutaneous blood flow of hind paw.

1.5.2 Specific aim 2: Determine the effect of surgical sympathectomy on the cutaneous blood flow of contralateral hind paw after injections of capsaicin

After the surgical sympathectomy, it is expected to eliminate the sympathetic component and there is no competitive effect from sympathetic activity. Then after the injection of the inflammatory chemical on the ipsilateral hind paws, DRR will be produced and transmitted to the contralateral side to cause the significant increase in cutaneous blood flow of contralateral hind paw. It is hypothesized that the contralateral post-injection cutaneous blood flow of the group with sympathectomy is significant higher than that of the control group.

CHAPTER 2

METHODS

All procedures used in this study were approved by the Animal Care and Use Committees of University of Texas at Arlington and followed the guidelines for the treatment of animals of the International Association for the Study of Pain (Zimmermann, 1983).

2.1 Subjects

Twenty four adult male Sprague-Dawley rats, over eight-month old, were involved in this study. All animals were from animal breeding colony from the University of Texas at Arlington.

2.2 Chronic surgical sympathectomy

Surgical sympathectomies were performed 7 days prior to experimental recording. Sympathectomies were performed in Sprague-Dawley male rats under pentobarbital anesthesia (50mg/kg). The sympathetic chain was exposed by a transperitoneal approach (Lin, Zou, Fang, & Willis, 2003b). The sympathetic paravertebral ganglia L2-L6 were removed bilaterally, since they are often fused together (Baron, J盲nig, & Kollmann, 1988). For more illustration, see appendix I.

In identifying levels of sympathetic ganglia, the most reliable landmark was found to be the left renal artery which runs near the L2 ganglion. The L5 ganglion was usually located just rostral to the bifurcation of the descending aorta. (Sun, Heung, Kwangsup, & Jin, 1993)

2.3 Animal preparation

Sprague-Dawley rats were anesthetized by sodium pentobarbital (50 mg/kg i.p.) before surgery. The depth of anesthesia was confirmed by the withdrawal responses to tail pinch. Continuous administration of anesthesia (5 mg/ml pentobarbital at rate of 1.2 ml/h, i.v.) during recording was accomplished by a catheter placed in the jugular vein. Tracheotomy was performed to monitor rat's respiration rate. Laminectomy was performed to expose the spinal cord from L1 to S1. The skin flips

were used to form a pool with warm mineral oil to prevent the spinal cord from drying out. Then ventral root L4 was identified according to the exit of spinal nerve L4 and the movement of hind paw when it was stretched. Then it was placed on a bipolar electrode. Before stimulating, animals were paralyzed with pancuronium (1 mg initially and 0.05mg/ml continuously at rate of 1.2 ml/h, i.v.) to prevent muscle contraction and ventilated artificially. The stimulating parameters were 10 Hz, 10 V, and 1 ms for 30s, 1 min and 2 min based on our preliminary study.

2.4 Blood flow recording

The paws of rat were stabilized by clay. The blood flow of both ipsilateral and contralateral were recorded by Laser Doppler Imager (PeriScan PIM II, Laser Doppler Perfusion Imager; Perimed AB; Stockholm, Sweden) scanning the whole area of paws. There were two groups, one with surgical sympathectomy, one sham group without actually sectioning sympathetic ganglia. Those two groups have the same procedures as shown in Figure 4. The first 10 images served as baseline. After base line, rats received ventral root L4 electrical stimulation with different parameters; then, rats received subcutaneously injections of capsaicin (0.1%, 10μl) on the ipsilateral side. After 10 images, another same set of electrical stimulations were applied.

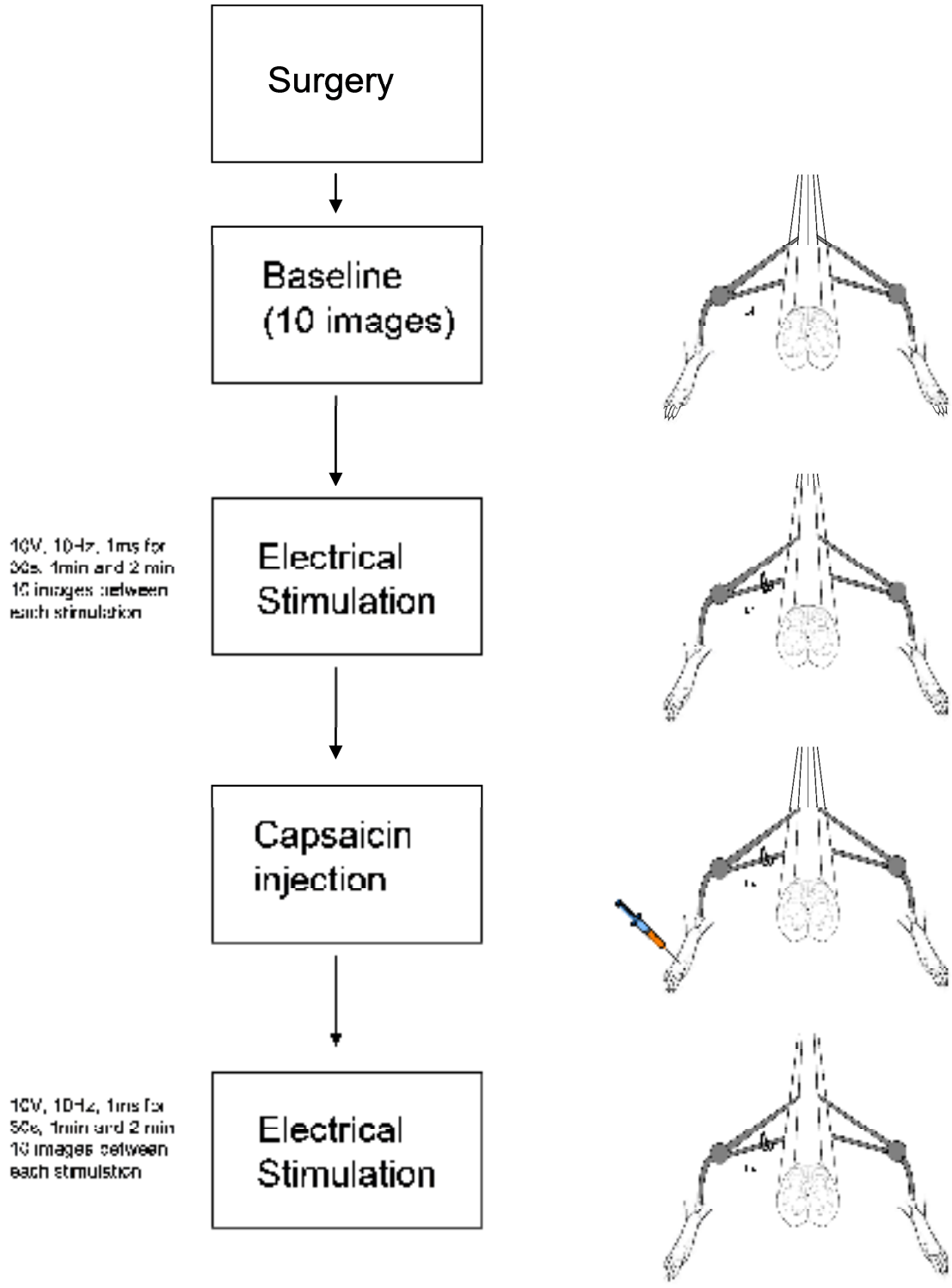


Figure 2.1 Schematic illustration of Experimental procedure

2.5 Glyoxylic acid staining of sympathetic fibers

Animals were euthanized by an overdose of pentobarbital after recording, and the femoral vein was dissected immediately. The dissected vein slip was immersed in 0.1 M phosphate buffer (pH 7.0) immediately, then those connecting tissues and fat were removed. Vein was cut longitudinally, and dipped in 2% glyoxylic acid (pH 7.0, 0.1M phosphate buffer) for 3 times. Then tissue was mounted on the slide with endothelium side facing up. The slide was incubated in a 100 ^{o}C oven for 4 min after it was air-dried. Catecholamine-positive nerve fibers were examined under a fluorescent microscope (FT460 nm). (Lin, Zou, Fang, & Willis, 2003a)

2.6 Data analysis

For the hypothesis I, the blood perfusion raw data were transformed to difference data by subtracting blood perfusion of each image from its previous image. Then single sample t-test was used to test the whether difference at each electrical stimulation was significantly lower than 0. Repeated measure ANOVA was also used to analyze the raw data and transformed average data, the results were presented in appendix II.

For the hypothesis II, ANCOVA was used to test whether contralateral post-injection cutaneous blood perfusion (average blood perfusion of 5 images after capsaicin injection) of sympathectomized group was is significant higher than the sham group. The average blood perfusion of 5 images right before capsaicin injection served as covariate.

The post-hoc used was bonferroni, if needed. Significance was set at $p = 0.05$.

CHAPTER 3

RESULTS

Twenty four rats were used in this study. Twelve rats for each group, sympathectomized group and sham group. One rat was deleted from sham group because of the rat died during the recording. Rats were eight-month old or older, and weight ranged from 402 g – 610 g.

3.1 Hypothesis I

For the first hypothesis, I expected that the electrical stimulation of ventral root L4 would reduce the blood perfusion of the paw. Because the sympathetic preganglionic fibers in the ventral root can be activated by the electrical stimulation, thereby release norepinephrine to constrict the blood vessel. Three different electrical stimulations were applied before and after capsaicin injection. The parameters were 10v, 1ms, 10 Hz for 30s, 60s or 120s. I analyzed these differences by subtracting the blood perfusion of each image from its previous image. So, the change at the stimulating image was expected to be negative if the electrical stimulation decreased the blood perfusion. The raw blood perfusion trend was shown for both sham group and sympathectomized group in figure 3.1 and figure 3.2. The transformed differences were shown for both sham and sympathectomized group in figure 3.3 and figure 3.4. The trends of both raw data and differences are very similar for both sham and sympahtectomized groups.

3.1.1 Sham group

For the sham group ipsilateral side, the differences at the 3rd electrical stimulation before capsaicin injection and 1st and 3rd electrical stimulation after capsaicin injection were significantly lower than 0 by using one-tailed single sample t test (t (10) = 0.051; t (10) = 0.0405; t (7) = 0.014). For the contralateral side, the differences at the 2nd and 3rd electrical stimulation before capsaicin injection and 2nd and 3rd electrical stimulation after capsaicin injection were significantly lower than 0 (t (10) = 0.0485; t (10) = 0.032; t (10) = 0.0195; t (7) = 0.0095). Table 3.1

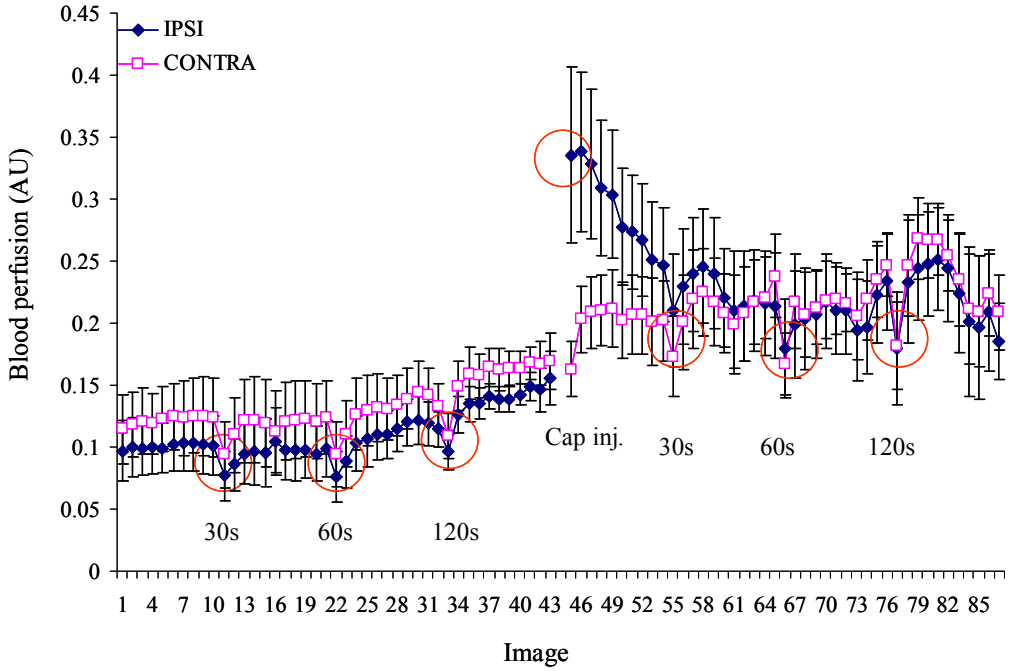


Figure 3.1 The trend of raw data for sham group. The circles indicate the stimulations in order from left to the right: 3 electrical stimulations before capsaicin injection (10v, 1ms, 10Hz for 30s, 60s or 120s); capsaicin injection; 3 electrical stimulations after capsaicin injection (10v, 1ms, 10Hz for 30s, 60s or 120s).

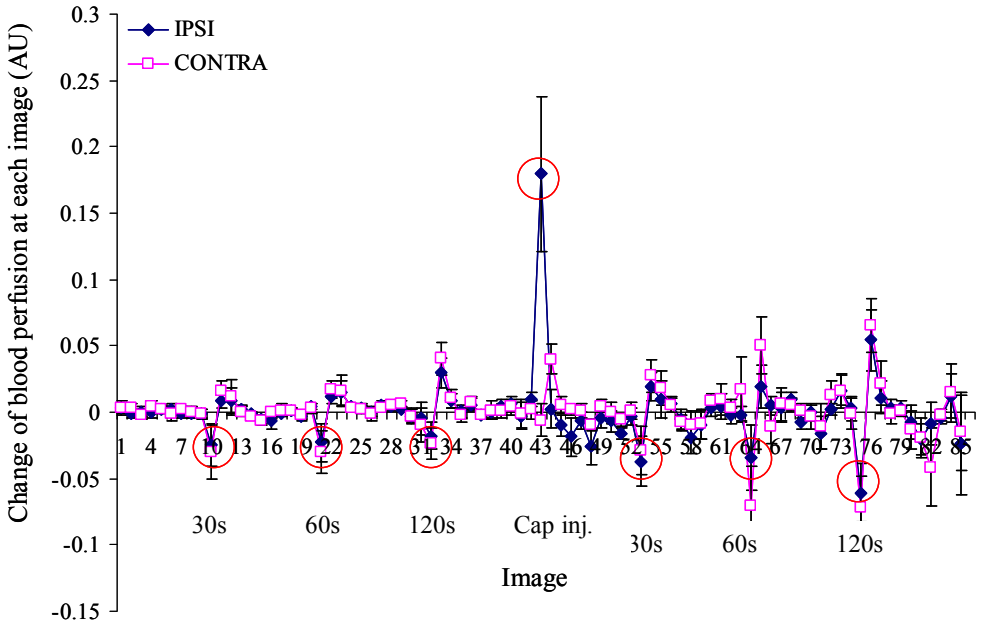


Figure 3.2 The trend of differences for sham group. The circles indicate the stimulations in order from left to the right: 3 electrical stimulations before capsaicin injection (10v, 1ms, 10Hz for 30s, 60s or 120s); capsaicin injection; 3 electrical stimulations after capsaicin injection (10v, 1ms, 10Hz for 30s, 60s or 120s).

Table 3.1 Significance of differences at different electrical stimulations for sham group. Upward arrow indicates significant increase in blood perfusion; downward arrow indicates significant decrease in blood perfusion. (E.S.: electrical stimulation)

Side	1st E.S. (30s)	2nd E.S. (60s)	3rd E.S. (120s)	Capsaicin injection	1st E.S. (30s)	2nd E.S. (60s)	3rd E.S. (120s)
Ipsilateral			0.051	↑	↓		↓
Contralateral		↓	↓			↓	↓

3.1.2 Sympathectomized group

For the sympathectomized group ipsilateral side, the differences at the 1st and 2nd electrical stimulations before capsaicin injection and 1st, and 2nd electrical stimulations after capsaicin injection were significantly lower than 0 (t (11) = 0.021; t (11) = 0.006; t (11) = 0.001; t (11) = 0.0305;). For the

contralateral side, the differences at the 1st , 2nd and 3rd electrical stimulations before capsaicin injection and 1st, 2nd and 3rd electrical stimulations after capsaicin injection were significantly lower than 0 (t (11) = 0.0205; t (11) = 0.00505; t (11) = 0.053; t (11) = 0.0015; t (11) = 0.009; t (11) = 0.0275). Table 3.2

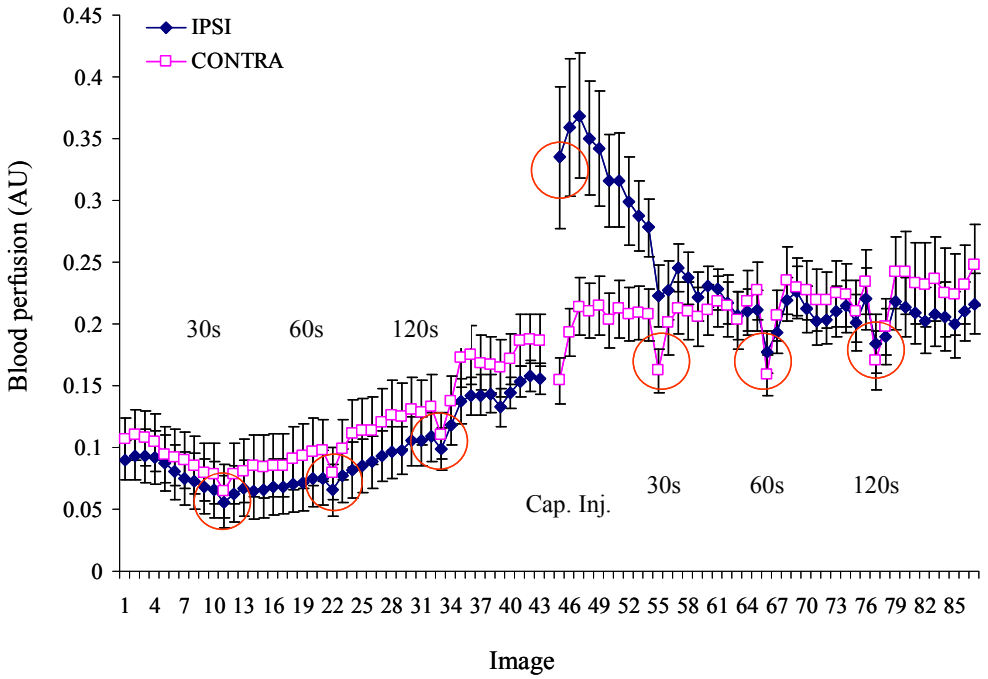


Figure 3.3 The trend of raw data for sympathectomized group. The circles indicate the stimulations in order from left to the right: 3 electrical stimulations before capsaicin injection (10v, 1ms, 10Hz for 30s, 60s or 120s); capsaicin injection; 3 electrical stimulations after capsaicin injection (10v, 1ms, 10Hz for 30s, 60s or 120s).

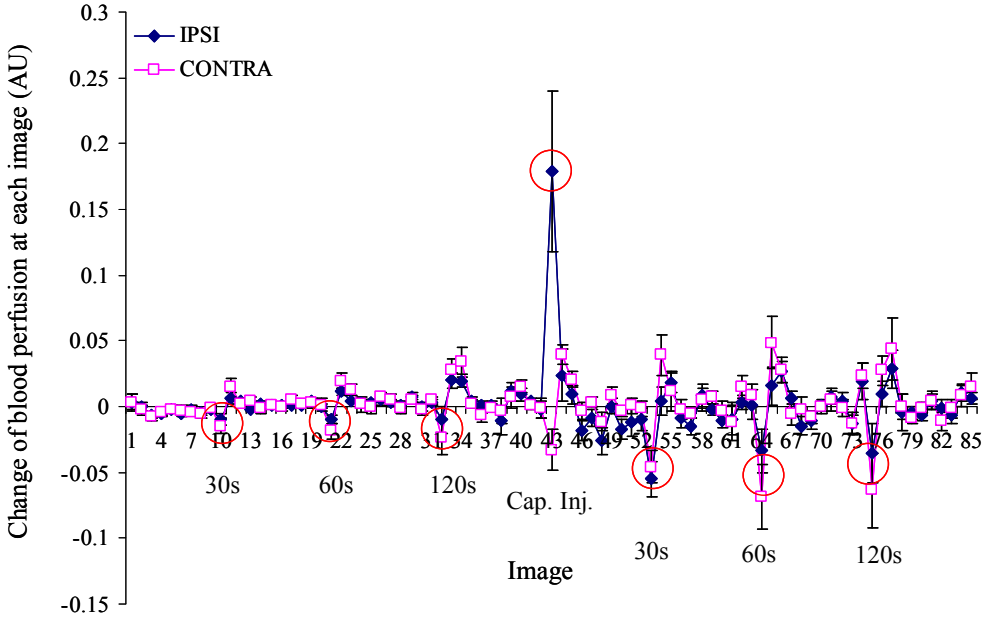


Figure 3.4 The trend of differences for sham group. The circles indicate the stimulations in order from left to the right: 3 electrical stimulations before capsaicin injection (10v, 1ms, 10Hz for 30s, 60s or 120s); capsaicin injection; 3 electrical stimulations after capsaicin injection (10v, 1ms, 10Hz for 30s, 60s or 120s).

Table 3.2 Significance of differences at different electrical stimulations for sympathectomized group. Upward arrow indicates significant increase in blood perfusion, downward arrow indicates significant decrease in blood perfusion. (E.S.: electrical stimulation)

Side	1st E.S. (30s)	2nd E.S. (60s)	3rd E.S. (120s)	Capsaicin injection	1st E.S. (30s)	2nd E.S. (60s)	3rd E.S. (120s)
Ipsilateral	↓	↓		↑	↓	↓	
Contralateral	↓	↓	0.053	↓	↓	↓	↓

3.1.3 Comparisons between different paws and among different electrical stimulations

A 2 by 6 repeated measure ANOVA was used to prove if there was any difference existing in the effect of electrical stimulation on the blood perfusion between two paws or any differences among different electrical stimulations. For sham group, there was no difference between ipsilateral paw and

contralateral paw, F (1, 6) = 3.924, p = 0.095. There was significant difference among different stimulations, F (5, 30) = 3.924, p = 0.037; but the bonferroni post hoc didn't show the significance of pairwise comparison. There was no interaction between the different paws and different stimulations, F (5, 30) = 2.339, p = 0.066. For sympathectomized group, there was no difference between ipsilateral paw and contralateral paw, F (1, 10) = 3.048, p = 0.111. There was no significant difference among different stimulations, F (1.695, 16.953) = 2.968, p = 0.085. There was an interaction between the different paws and different stimulations, F (1.952, 19.524) = 3.959, p = 0.037, however the bonferroni post hoc didn't show the significance of pairwise comparison.

Two 2 by 6 mixed ANOVAs was performed to see if the effect of electrical stimulation differs in two groups on both ipsilateral and contralateral sides. There was no group difference on ipsilateral side, F (1, 16) = 1.525, p = 0.235; there was no group difference on contralateral side, F (1, 17) = 0.573, p = 0.460.

3.2 Hypothesis II

3.2.1 Comparisons of change of contralateral blood perfusion by capsaicin injection between two groups

For hypothesis II, I expected that effect of DRRs on the contralateral side should be more obvious after elimination of sympathetic component, thus the increase of contralateral blood perfusion would manifest. It was hypothesized that the blood perfusion after capsaicin injection for the sympathectomized group was significantly higher than the sham group. I used two different ways to analyze the data.

First, I took the average of the blood perfusion of 5 images before capsaicin injection and 5 images after capsaicin injection. Then the ANCOVA was used to compare the average blood perfusion after capsaicin injection between two groups using the average blood perfusion before capsaicin injection as the covariate. The result indicated there was no difference between the two groups in the blood perfusion after capsaicin injection, F (1, 20)=0.641, p =0.433.

Second, I got the differences for both groups by subtracting the blood perfusion of the image right after capsaicin injection from that of the image right before the injection. Then an independent t-test

was used to test whether the change of contralateral blood perfusion caused by capsaicin injection was higher for sympathectomized group that sham group. It turned out there was no difference in the capsaicin-caused change in blood perfusion between the two groups, t (23) =1.352, p =0.191.

3.2.2 The effect of capsaicin injection on the blood perfusion

For both groups, the ipsilateral blood perfusion increased significantly after capsaicin for both sham group (t (10) = 2.993, p =0.013) and sympathectomized group (t (11) = 3.279, p = 0.007). However, the contralateral blood perfusion tended to decrease when ipsilateral side was injected with capsaicin, t (10) = -0.213, p = 0.836 for sham group and t (11) = -2.015, p = 0.069 for sympathectomized group.

3.3 Histology

Glyoxylic acid staining was performed to verify degeneration of sympathetic nerve on the blood vessel after blood perfusion recording. Figure # shows the comparison between the sham group and sympathectomized group.

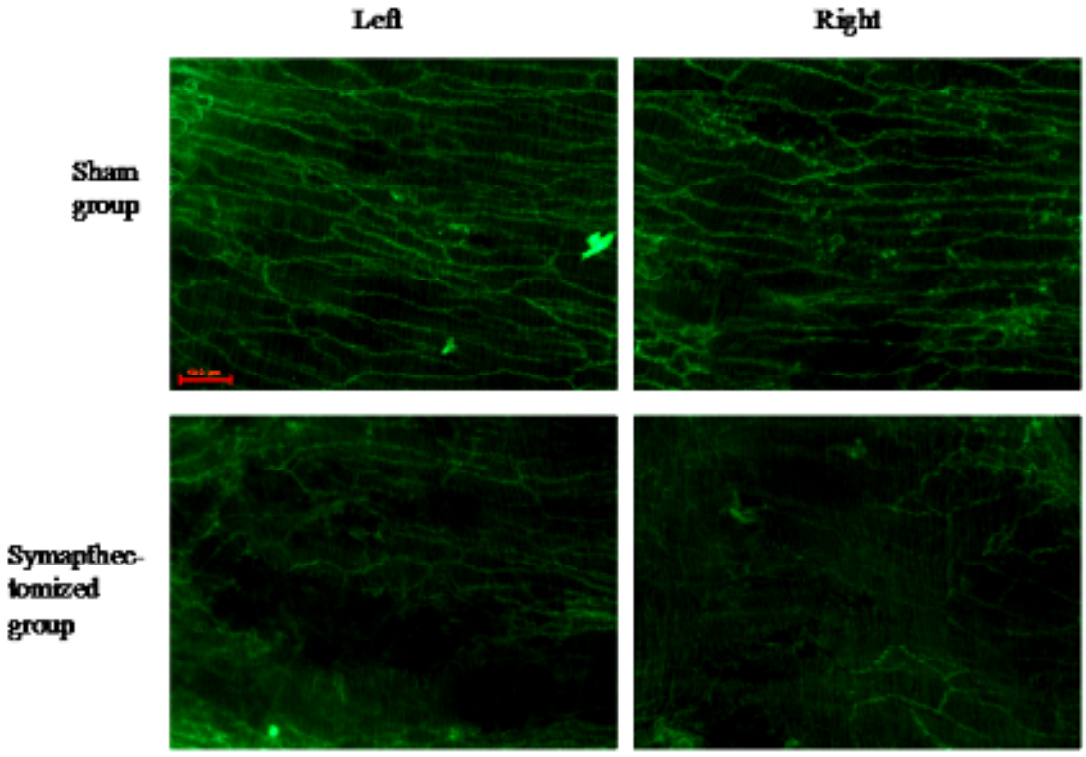


Figure 3.5 Comparison of histology between sham group and sympathectomized group

CHAPTER 4

DISCUSSION

This study aimed to investigate whether the elimination of competition from sympathetic activity could unveil the effect of DRRs on the contralateral side of the hind paw, the increase of contralateral blood perfusion would manifest after chemical stimulation, capsaicin injection.

4.1 Hypothesis I

The results indicated that the activation of sympathetic nerve did constrict blood vessel and caused the decrease of blood perfusion on both ipsilateral and contralateral hind paws. According to the anatomical structure of the sympathetic nervous system, the preganglionic fibers, sent out from the spinal cord, project to the sympathetic chain where the postganglionic neurons reside. At there, the preganglionic fibers can synapse with the postganglionic neurons at the same level where they originate from, they can also synapse with postganglionic neurons at other levels by travelling up or down along the sympathetic chain (Iversen et al., 2000). In this study, I stimulated L4 ventral root, which has the sympathetic preganglionic fibers projecting to lumbosacral sympathetic ganglia. And lumbosacral sympathetic ganglia projects postganglionic fibers to many organs of the lower trunk including the hind limb (Wang, Holst, & Powley, 1995b). So, when the L4 ventral root was electrically stimulated, the postganglionic fibers innervating the hind limb were also activated to constrict the blood perfusion of hind paw. Sympathetic nervous system has more diffuse effect than parasympathetic nervous system (Wang, Holst, & Powley, 1995a). There are several anatomical studies indicated that the contralateral projection of sympathetic preganglionic neurons. (Faden & Petras, 1978) proved that in dog, contralateral projection existed for lumbar ganglia, not for thoracic ganglia by studying the retrograde axonal transport of horseradish peroxidase. Such contralateral projection has also been proved in puffer fish (Funakoshi, Abe, Rahman, & Kishida, 1997). There is no direct evidence that the sympathetic preganglionic neurons also project contralaterally in rats, however, it is possible that this phenomenon also exists in rats.

Moreover, according to (Baron et al., 1988), the lumbar sympathetic ganglia of both sides are often fused, so activation of ipsilateral sympathetic preganglionic fibers may activate postganglia of both sides. In this study, the blood perfusion of both sides always changed together with the electrical stimulation.

However, for the sympathectomized group, I also observed this vasoconstriction phenomenon by electrical stimulation of L4 ventral root. There was no difference in the blood perfusion between the sympathectomized group and sham group. One possible explanation for this is that the sympathetic chain and ganglia were not taken off completely, so the residuals can still constrict the blood vessel when they were activated. However, I would still expect smaller decrease in blood perfusion at electrical stimulations for sympathectomized group than sham group, after the removal a big portion of the sympathetic chain and ganglia (appendix II). Another possible explanation is that when the preganglionic fibers in L4 ventral root were stimulated, some of the preganglionic fibers that innervate the adrenal gland were activated to stimulate adrenal gland to release norepinephrine, thus constrict the blood vessel systemically. If that's the case, it will take some time for norepinephrine to get into the circulation and get to the target. However, in this study, the decrease of blood perfusion took place immediately when the electrical stimulation occurred. There may be some other unknown mechanism causing this decrease of blood perfusion in sympathectomized group.

4.2 Hypothesis II

In this study, the capsaicin injection could immediately increase the blood perfusion of the ipsilateral, not the contralateral hind paw, which is consistent with the previous study in our lab. Capsaicin can bind to TRPV1 receptor on nociceptors, to activate the nerve terminal to release neuropeptides, such as substance P and CGRP. SP and CGRP can further increase vasodilation, thus then increase of blood perfusion (Cortright & Szallasi, 2009). According to the DRR theory as summarized in introduction, the activation of nociceptors by capsaicin could eventually result in the production of DRRs. And DRRs are able to travel bilaterally (Peng, Kenshalo, & Gracely, 2003). So the contralateral blood perfusion was expected to increase after ipsilateral capsaicin injection. However, I didn't observe the increase of blood perfusion for the contralateral hind paw. In contrast, the blood perfusion of contralateral

hind paw tended to decrease at the moment of capsaicin injection. Sympathetic nervous system can be activated by incoming noxious stimuli (Korim, McMullan, Cravo, & Pilowsky, 2011). The capsaicin injection was able to induce sympathetic activity. For the ipsilateral side, the strong vasodilation by capsaicin injection surpasses sympathetic, while for contralateral side, the sympathetic vasoconstriction counteract with the DRRs-induced vasodilation. However, in this study, increase of contralateral blood perfusion was not observed even after the removal of supposed competitive component. This indicates that DRR is too weak to evoke the inflammation response on the contralateral side.

4.3 Conclusions and future directions

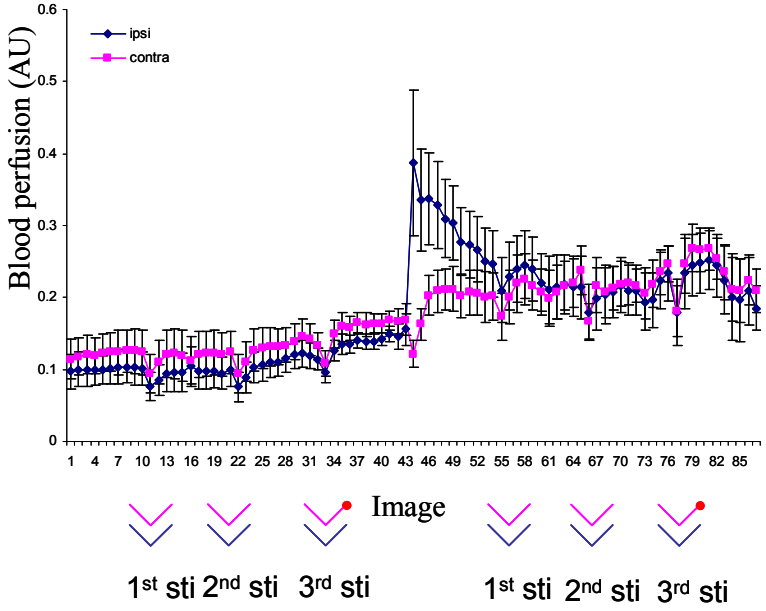
The removal of sympathetic component couldn't enhance the blood perfusion of contralateral paw after capsaicin injection compared with that of sham group. The DRR by itself is too weak to induce the neurogenic inflammation on the contralateral side. The stimulation of L4 ventral root had vasoconstricting effect on both sides, which was also seen in the sympathectomized group. This may be due to the incomplete removal of sympathetic chain. So, for the future direction, I would like to try the chronic chemical sympathectomy, to see the difference between the results of those two different methods.

APPENDIX A

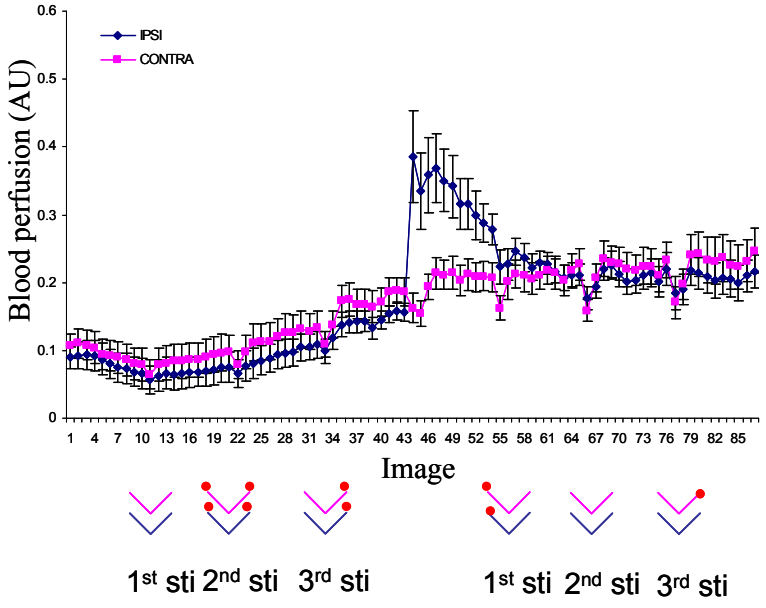
OTHER ANALYSES

1. Repeated ANOVA was used to analyze changes of blood perfusion of consecutive three images for each electrical stimulation: the image right before electrical stimulation, the image at electrical stimulation, the image right after electrical stimulation. Bonferroni was chosen as the post-hoc test and significant level was set at 0.05.

a. Sham group

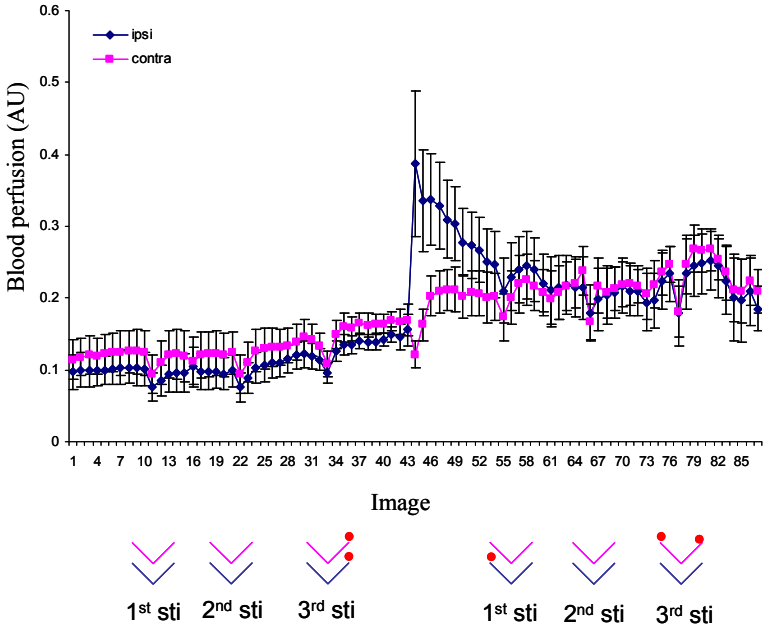


b. Sympathectomized group

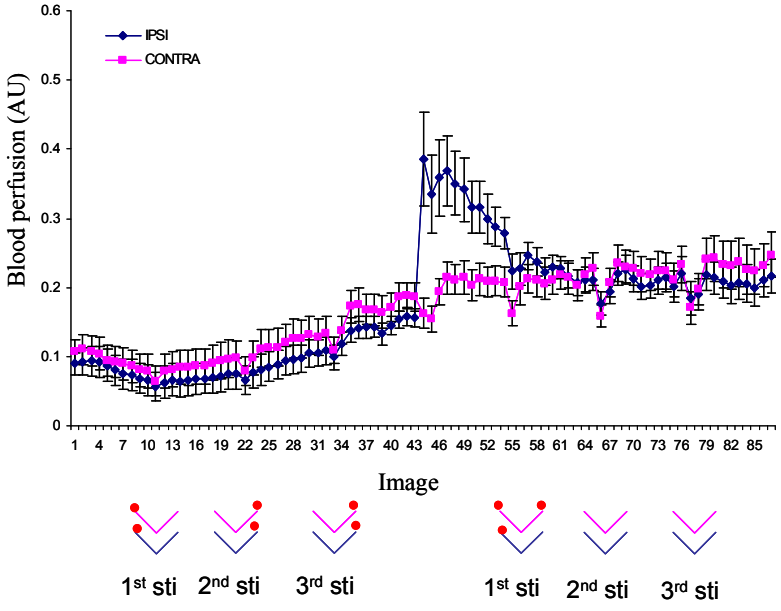


2. For this analysis, instead of using the blood perfusion of image right before and after electrical stimulation, I took the average of blood perfusion of 5 images before and after electrical stimulation. And as aforementioned, the repeated ANOVA was used to analyze this data.

a. Sham group



b. Sympathectomized group



The downward arrows at the bottom of each figure represent the consecutive three images around each stimulation. Blue color represents ipsilateral side, pink color represents contralateral side. Red dots at left indicates the blood perfusion of the image was significantly higher than the blood perfusion at the stimulation, while the red dots at right indicates the blood perfusion of the image after stimulation was significantly higher than the blood perfusion at the stimulation.

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BIOGRAPHICAL INFORMATION

Ailing Li was born in China. She got her bachelor's degree in biotechnology and master's degree in environmental zoology in Lanzhou University, China. She came to the U.S. in 2009 to study in the health psychology and neuroscience program at the University of Texas at Arlington. Her major interest is in the pain field, especially neurogenic inflammation. She studies the factors involved in neurogenic inflammation, like the dorsal root reflex and sympathetic nervous system.