THE CONTRIBUTION OF THE DORSAL ROOT REFLEX AND THE SYMPATHETIC NERVOUS SYSTEM IN FORMALIN-EVOKED NEUROGENIC INFLAMMATION

by

LARA A. KACHLIC

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ABSTRACT

THE CONTRIBUTION OF THE DORSAL ROOT REFLEX AND THE SYMPATHETIC NERVOUS SYSTEM IN FORMALIN-EVOKED NEUROGENIC INFLAMMATION

Lara A. Kachlic, M.S.

The University of Texas at Arlington, 2008

Supervising Professor: Dr. Yuan B. Peng

The present study investigated the role of dorsal root reflex (DRR), which is characterized as antidromic firing via primary afferent fibers towards the periphery, and the sympathetic nervous system in neurogenic inflammation. Neurogenic inflammation is defined as inflammation that is caused by substances released from sensory nerve terminals (Willis Jr., 1999). The first objective of the study was to determine bilateral blood perfusion, a measure of redness, one of the cardinal signs of inflammation, following a formalin injection with the use of laser Doppler imaging. The hypothesis stated that there would be a bilateral increase in blood perfusion, following formalin. It was found that following an injection of an inflammatory agent, formalin, under pentobarbital general anesthesia, there was an ipsilateral increase in blood perfusion,

but not in the contralateral paw. The lack of change of blood perfusion in the contralateral paw may have been due to the antagonistic effects of vasodilatation by the DRR and the vasoconstrictive effects of the sympathetic nervous system. The second objective of the study was to determine the role of DRR in blood perfusion in neurogenic inflammation. The hypothesis stated that by blocking DRR transmission to the ipsilateral side, there would be a decrease in blood perfusion to the ipsilateral side, and there would be no change in blood perfusion to the contralateral side, following formalin. The left sciatic nerve was transected following local lidocaine application, and formalin was injected into the left hind paw. The results showed a unilateral increase in blood perfusion in the ipsilateral side following formalin injection. This indicates that local axonal reflex may contribute to neurogenic inflammation. Additionally, the sympathetic nervous system may contribute to this inflammation indirectly through the release of norepinephrine in the periphery. The third objective of this study was to determine the role of the sympathetic nervous system in neurogenic inflammation. The hypothesis stated that by eliminating the sympathetic nervous system through chemical sympathetic block, there would be a greater bilateral increase in blood perfusion following formalin injection. Using the guanethidine chemical sympathetic block model, formalin injection in the sciatic transected animals caused an increase in blood perfusion in the ipsilateral paw, but not the contralateral paw. This indicates that local axonal reflex may play a larger role in neurogenic inflammation than previously expected.

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INTRODUCTION

Complex regional pain syndrome (CRPS), also referred to as sympathetically maintained pain, is a condition not clearly understood (Birklein and Schmelz, 2008). Therapies that target the sympathetic nervous system through elimination or chemical blockade have been under taken, unfortunately with variable results. Additionally, neurogenic inflammation and neuropeptides associated with this inflammation have also been implicated in the maintenance of this pain (Birklein and Schmelz, 2008). Neurogenic inflammation is defined as an inflammatory state that is caused by the release of substances from sensory nerve terminals (Willis Jr., 1999). There are several processes that contribute to the four cardinal signs of neurogenic inflammation: pain, redness, edema, and increased temperature. Dorsal root reflex (DRR), first described by Gotch and Horsley (1891), has been shown to contribute to the redness and edema associated with this type of inflammation. However, during neurogenic inflammation, the sympathetic nervous system is also activated, contributing a possible antagonistic effect in terms of blood perfusion to the injury through the release of norepinephrine in the periphery, causing vasoconstriction of the peripheral venules. In addition to the DRRs and the sympathetic nervous system, the inflammatory response, specifically the bradykinins and prostaglandins that are released following injury, seems to perpetuate the activation of DRR and the sympathetic nervous system (Kandel, Schwartz, and

Jessell, 2000). The purpose of the present study was to determine what unique roles the DRR and the sympathetic nervous system may play in the change of blood perfusion of neurogenic inflammation.

1.1 Dorsal Root Reflex

Dorsal root reflex is characterized as an antidromic firing via primary afferent fibers toward the periphery (Barron and Matthews, 1935). After an injury occurs in the periphery, the nociceptors are activated and nociceptive action potentials are sent via primary afferent fibers to the spinal cord. Studies have shown that once these signals reach the spinal cord, DRR generation occurs when the nociceptive action potentials cause the release of glutamate from the primary afferent fibers. The primary afferent fibers make synapse to a GABAergic interneuron in the dorsal horn of the spinal cord, which causes the release of GABA (Willis, 1999; Eccles and Schmidt, 1963). The GABA then binds to $GABA_A$ receptors on the primary afferent terminal and causes an efflux of chloride ions, causing depolarization of the primary afferent (Cervero and Laird, 1996; Rudomin and Schmidt, 1999; Willis, 1999). This, in turn, generates an action potential down the primary afferent fiber towards the periphery (Cervero and Laird, 1996; Willis, 1999; Cervero, Laird, and Garcias-Nicas, 2003). Peng et al. (2001) showed that when GABA was applied to the exposed spinal cord, there was a significant increase in DRR activity. This indicated that $GABA_A$ receptors are involved in the generation of DRRs. Lin, Jing, and Willis (1999) found that, when treated with bicuculline, a $GABA_A$ antagonist, after a capsaicin injection, DRR activity was almost completely eliminated.

When GABA is released in the spinal cord, DRR is generated and transmitted back to the periphery via primary afferent fibers, contributing to vasodilatation and plasma extravasation through the release of neuropeptides (Lam, Ferrell, and Scott, 1993; Kessler, Habelt, Averbeck, Reeh, and Kress, 1999; Alvarez et al., 1988). Bayliss (1901) found that stimulation of the dorsal root caused vasodilatation in the periphery. He proposed that fibers involved were not efferent fibers, but instead primary afferent fibers. Hilton and Marshall (1980) confirmed Bayliss's findings, and in addition, proposed that the vasodilatation is due strictly to activation of the primary afferents, and not through activation of the sympathetic or somatic efferent fibers. In addition, Pinter and Szolcsanyi (1995) found that stimulation of C fibers in the dorsal root contribute to plasma extravasation in the periphery; however, stimulation of Aδ fibers produced vasodilatation, but not plasma extravasation. Rees, Sluka, Westlund, and Willis (1994) found that activity of DRRs in sympathectomized animals was the same as intact animals, confirming the findings of Hilton and Marshall (1980). However, Wang et al. (2004) proposed that the sympathetic fibers may have modulatory effects on the DRR. Using electrophysiological techniques, this study found that, following a capsaicin injection, DRRs were reduced in rats that had surgical sympathectomy. Additionally, DRRs were greatly reduced in sympathetically intact rats that were given terazosin, an α_1 antagonist.

Previously, it was unclear as to which primary fibers conduct DRRs back to the periphery. However, through the use of electrophysiological techniques, Sluka, Rees, Westlund, and Willis (1995) and Lin, Jing, and Willis (2000) found that Aδ and C fibers

individually conduct these signals back to the periphery. Using conduction velocities, Sluka et al. (1995) found that in articular fibers that Aδ and C fibers conducted DRRs to the site of injury in an arthritis model. Lin et al. (2000) found that following capsaicin injection into the hind paw of rats, there was an increase in DRR activity in Aδ and C fibers, but this increase did not occur in Aβ fibers. However, Garcia-Nicas, Laird, and Cervero (2001) found that stimulation of Aβ fibers in fact do evoke DRRs, thus contributing to vasodilatation. These findings indicate that the physiology of DRR still remains unclear.

Several studies have looked at the release of neuropeptides from different primary afferent terminals that may contribute to plasma extravasation and vasodilatation (Kilo, Harding-Rose, Hargreaves, and Flores, 1997; O'Brien, Woolf, Fitzgerald, Lindsay, and Molander, 1989; Alvarez et al., 1988; Ishida-Yamamoto, Senda, and Tohyama, 1989). Alvarez et al. (1988) found, through immunocytochemistry, that substance P and CGRP are located in the terminals of free nerve endings. The main limitation to this study was that the stain used had a higher affinity for CGRP. Ishida-Yamamoto et al. (1989) also looked at CGRP release from primary afferent fibers, and found that CGRP was most likely released from C fibers. The authors also indicate that CGRP is released also from Aδ fibers, but to a lesser degree than C fibers.

1.2 The Sympathetic Nervous System

The sympathetic nervous system becomes activated when primary afferent fibers send nociceptive action potentials to the spinal cord. Once in the spinal cord, the

primary afferents make direct synapse to the preganglionic fibers in the intermediolateral gray matter of the spinal cord (Hofstetter, Card, and Olson, 2005). The axons of the preganglionic fiber then exit the spinal cord via the ventral horn (Figure 1). They separate from the somatic motor neuron in small bundles called white rami, and make synapse to the postganglionic neuron in the ganglia of the sympathetic chain, which lies outside the central nervous system. The largely unmyelinated axons of the postganglionic fibers exit the ganglia in bundles called grey rami, and continue onto their respective target.

When the sympathetic nervous system is activated by nociceptive signals, norepinephrine is released in the periphery, causing vasoconstriction. The effect the sympathetic nervous system has on DRR during neurogenic inflammation is not clearly understood. The sympathetic nervous system may have a modulatory effect on the inflammatory agents. For example, Miao et al. (1996) found that in sympathectomized rats, there was a delay of extravasation, following infusion of bradykinins in the knee joint, indicating that sympathetic postganglionic neurons mediate plasma extravasation. Häbler, Wasner, and Jänig (1997) found that the vasoconstriction by the sympathetic nervous system and vasodilatation by CGRP interact to control blood flow. This study also shows that vasodilatation seems to override sympathetic vasoconstriction; however, this is not the case at small-diameter primary afferents. Further research is needed in order to fully understand the role of the sympathetic nervous system in neurogenic inflammation.

1.3 Neurogenic Inflammation

In addition to the sympathetic nervous system and the DRR, the inflammatory agents, such as bradykinins and prostaglandins, that are released upon injury have also been shown to contribute to the edema and redness associated with neurogenic inflammation. When an injury occurs in the periphery, bradykinins and prostaglandins are released. This, in turn, causes nociceptors to become activated, releasing substance P and calcitonin-gene related peptide (CGRP), which have been shown to cause plasma extravasation and vasodilatation, respectively (Kandel et al., 2000). When substance P is released, this causes degranulation of the mast cells, and thus the release of histamine, serotonin, and additional bradykinins and prostaglandins from these cells (Barnes et al., 1986). Brain and Williams (1989) found that histamine and serotonin antagonists reduced edema caused by the release of substance P. Additionally, when substance P is released, this causes plasma extravasation at the venules, and is thought to reach the venules through diffusion (Holzer, 1998). When degranulation of the mast cells occurs, this causes the release of additional bradykinins and prostaglandins, which in turn causes activation of nociceptors. This causes additional DRRs to be sent to the periphery, and additional release of substance P. The termination of this loop is unclear; however, dilution by diffusion and enzymatic breakdown have been implicated as possible explanations for the termination of neurogenic inflammation (Holzer, 1998). In addition, Holzer also suggests an "internalization of the peptide-receptor complexes" occurs, which could lead to desensitization of substance P at the mast cells (1998, p. 8). CGRP is also released when DRRs are sent to the periphery in neurogenic

inflammation. This neurotransmitter is mainly released from Aδ fiber terminals, but some research has shown it to be released from C fibers also (Ishida-Yamamoto et al., 1989). When CGRP is released from the primary afferent terminals, this causes vasodilatation of the arterioles (Holzer, 1998). Gibbins, Furness, Costa, MacIntyre, Hillyard, and Girgis (1985) found, using immunohistochemistry, that CGRP and substance P are cotransported from dorsal root ganglia, indicating that CGRP may have a modulatory effect on substance P. It is still not clear the role that substance P and CGRP may have on neurogenic inflammation, but also the interaction these neurotransmitters may have with each other.

1.4 Experimental hypotheses and objectives

The combined effects of DRR, the sympathetic nervous system, and the inflammatory agents that are released following injury contribute to the cardinal signs of neurogenic inflammation; however, their interaction in neurogenic inflammation are not clearly understood. Utilizing guanethidine, a common sympathetic block model (Johnson, Cantor, and Douglas, 1975) and formalin, an acute inflammatory model (Dubuisson and Dennis, 1977), the role of DRRs in neurogenic inflammation will be explored.

The ***central hypothesis*** was that neurogenic inflammation is influenced by the balance of DRRs and sympathetic activity. Increased DRRs will increase blood perfusion through the release of CGRP and substance P, whereas increased sympathetic activity will decrease blood perfusion by the release of norepinephrine. To determine

the balance of DRRs and sympathetic activity on blood perfusion, the following specific aims were addressed.

<u>Specific Aim 1:</u> To determine bilateral blood perfusion change evoked by formalin. A laser Doppler imager (LDI) was used to take images of both paws in an anesthetized rat. A baseline period of 10 images was used to compare the change in blood perfusion after a formalin injection in the left paw. It was expected that blood perfusion would increase in the ipsilateral paw due to local irritation and DRRs. Nociceptive signals evoked by formalin will propagate to the spinal cord, causing release of excitatory neurotransmitters, such as glutamate. Glutamate may activate the nearby primary afferent terminals directly (through NMDA and Non-NMDA receptors) or indirectly (through GABA released by GABAergic interneurons). This, in turn, will cause generation of DRRs, which would in turn cause the release of CGRP and substance P. Lin and Fu (1999) found that DRRs can be transmitted both ipsilaterally and contralaterally. In support of this evidence, the blood flow change in the contralateral paw will be determined by the balance between sympathetic activation (decreased blood perfusion due to the release of norepinephrine) and DRRs (increased blood perfusion due to release of CGRP).

<u>Specific Aim 2:</u> To determine the role of DRRs in blood perfusion change. To eliminate the role of the DRRs in neurogenic inflammation, the sciatic nerve was transected prior to formalin injection. Bilateral images were taken with the LDI. The results were expected to show less of an increase in blood perfusion to the ipsilateral side following formalin compared to results from Specific Aim 1, due to the fact that

DRRs will not reach the ipsilateral periphery due to nerve transection. An increase in blood perfusion was not expected on the contralateral side, due to the fact that DRRs would be blocked by the sciatic nerve transection and would not be transmitted to the contralateral side.

Specific Aim 3: To determine the contribution of the sympathetic nervous system in blood perfusion change. The same experiment from Specific Aim 1 was performed after undergoing a guanethidine-induced chemical sympathetic block. The subjects received a formalin injection, and images were taken throughout the procedure. It was expected that both paws would have increased blood perfusion following formalin injection. Without the influence of the sympathetic activity, DRRs will be a major contributor to bilateral vasodilatation. An additional sciatic nerve transection in the sympathetic block group was expected to have an increase of blood perfusion in the ipsilateral paw, but not the contralateral paw. Since the sympathetic nervous system will not be activated and DRRs will not be generated due to nerve transection, the contralateral paw was expected to remain the same in terms of blood perfusion. By accomplishing these specific aims, a differentiation of the roles played by sympathetic activity and DRRs on blood perfusion in neurogenic inflammation can be achieved. Understanding the role of the sympathetic nervous system and the DRR in neurogenic inflammation will give a better understanding of how these factors affect the physiological state during injury, and ultimately lead to the development of improved therapies for pain relief.

CHAPTER 2

METHODS

Subjects were maintained at the University of Texas at Arlington Animal Care Facility. Thirty-two adult (60 days old) male Sprague-Dawley rats were used as subjects, and were maintained on a 12 hour light:dark cycle. Prior to experiments, the animals were housed four to a cage and were allowed free access to food and water.

Animals were used and cared for according to the guidelines published by the Committee for Research and Ethical Issues for the study of pain (Zimmerman, 1983), and in accordance with the Institutional Animal Care and Use committee of the University of Texas at Arlington. Every measure was taken to limit the number of subjects used and to minimize the suffering of each subject.

2.1 Surgical Procedures

Subjects were anesthetized with an intraperitoneal injection of sodium pentobarbital (Sigma, 50 mg/kg). Level of anesthesia was assessed by lack of motor response to pinching of the tail and hind paws. The sciatic nerve was exposed for experimental manipulations. A tracheotomy and jugular vein cannulation were performed in order to monitor respiration and administer anesthesia at a constant rate, respectively.

2.1.1 Tracheotomy

The animal was placed in the supine position on the surgery table, in order to perform tracheotomy and jugular vein cannulation. A two centimeter longitudinal incision was made just superficial to the trachea. The muscles around the trachea were split in the midline and the trachea was exposed from the surrounding muscles and tissue. Nylon thread was placed under the trachea for better exposure. The cartilage of the trachea was then cut in half transversely to the trachea in order for tube insertion. A 9.5 centimeter long plastic tube (PE50) in length with a 3 millimeter diameter was inserted into the open trachea. The thread was double knotted to the trachea for stabilization purposes.

2.1.2 Jugular vein cannulation

A plastic anesthesia line (PE10), connected to a syringe of sodium pentobarbital (Sigma, 5 mg/ml), was used to maintain anesthesia intravenously through the jugular vein. The jugular vein was isolated from surrounding tissue, and nylon thread was placed under the vein for better insertion. A semicircular cut was made in order to insert the anesthesia tube. Using size 4 forceps, the vein was held open and the anesthesia line (PE10) tube was inserted into the jugular vein. The tube was tied to the vein, in order to hold the anesthesia line in place. Insertion was verified by the presence of blood in the anesthesia line after pulling back on the syringe of anesthesia. The anesthesia was attached to the anesthesia pump (Harvard apparatus, pump II) and rate was set at 1.2 ml per hour.

2.1.3 Sciatic nerve exposure and lidocaine application

This procedure was performed prior to tracheotomy and jugular vein cannulation. The left hind leg was shaved, and a three centimeter incision was made parallel to the hind leg. The muscles were cut and held back with a tissue retractor in order to expose the sciatic nerve. Once the sciatic nerve was located, the nerve was isolated from neighboring tissue. After baseline images and sympathetic block manipulations, the sciatic nerve was placed on an electrode and verified by establishing a visual and auditory signal of neuronal activity through the use of electrophysiological equipment. After verification, a small portion of cotton was placed on the nerve, and 0.2 milliters of lidocaine hydrochloride (Phoenix Pharmaceticals, 2%) was applied topically dropwise to the exposed nerve. After the lidocaine application, a period of 2 images (approximately 3.5 to 5 minutes) was allotted in order for the drug to take effect. The nerve was verified again to ensure the effects of the lidocaine. Then the nerve was cut and ten more images were taken with a laser Doppler imager (LDI).

2.2 Chemical sympathetic block

There are two different ways of performing a sympathetic block: chemically or surgically. For the present study, guanethidine (Research Diagnostics, Inc, 10 mg/kg) was used to temporarily block sympathetic activity (Gonzalez, Carmichael, Dostrovsky, and Charlton, 2005). After baseline images, an i.p. injection of guanethidine was given and 2 hours of images were taken in order for the drug to take effect.

2.3 Formalin

A subcutaneous injection of 0.05 milliliters of dilute formaldehyde (3%) was given in the plantar side of the left hind paw after baseline measurements and subsequent manipulations (Dubbuisson and Dennis, 1977). Due to the level of anesthesia, 3% formalin was chosen to ensure an inflammatory effect.

2.4 Laser Doppler imaging (LDI)

Following the surgical procedures (approximately 30-45 minutes after general anesthesia was administered), the animal was placed in a stereotaxic frame, and the legs were positioned so that both paws would have images taken simultaneously. The laser Doppler imager (Perimed AB, Periscan PIM II, Stockholm, Sweden), which is used to measure blood perfusion, was positioned above the paws. The intensity reading in the software (LDPI, 2.6) was set at 8.5 volts and the scanning area was set to cover both paws. When the imager parameters were set to capture both paws and the intensity reading was set, the imager was started through the software. Ten baseline images (each image took approximately 2 minutes to scan) were taken for all groups. After the baseline images, manipulations (chemical sympathetic block, application, sciatic nerve transection, and formalin injection) were made according to their experimental group. Images were taken following the manipulations, and the animals were sacrificed by overdose of sodium pentobarbital after the experiments.

2.5 Experimental Procedures

Table 1 shows the groups, measurements, and the number of animals used for each group: saline-no cut, saline-cut, guanethidine-no cut, and guanethidine-cut.

2.5.1. Experiment-Saline-No Cut

Experiment 1 tested the effects of formalin on blood perfusion. The subjects underwent a sciatic nerve exposure, tracheotomy, and jugular vein cannulation. After surgery, the animal was then immediately placed in the stereotaxic frame and positioned for images to be taken. After 10 baseline images were taken, the animal received an intraperitoneal injection of saline (1 mg/kg), in order to control for the intraperitoneal injection of the sympathetic block. After two hours of images were taken, the sciatic nerve was placed on the electrode and verified. After verification, a small portion of cotton was placed on the nerve and 0.2 milliliters of saline was applied drop wise to the nerve. Ten images were taken and then the subject received a formalin injection in the left hind paw. Images were taken for 60 minutes following the formalin injection.

2.5.2 Experiment 2-Saline-Cut

Experiment 2 tested the role the DRRs have on blood perfusion during inflammatory pain. The subjects were anesthetized and a sciatic nerve exposure, tracheotomy, and jugular vein cannulation were performed. Immediately following the surgical procedure, the subject was placed in a stereotaxic frame and ten baseline images were taken. After the baseline period, an intraperitoneal injection of saline (1 mg/kg) was given, and two hours of imaging was taken. Following the 2 hour period, the sciatic nerve was verified and lidocaine was applied to the exposed nerve. After the allotted time period for lidocaine to take effect, the nerve was cut. Ten images were taken and then the subject received a formalin injection in the left hind paw. Images were taken for 60 minutes following the formalin injection.

2.5.3 Experiment 3-Guanethidine-No Cut

Experiment 3 tested the effects of the sympathetic nervous system on blood perfusion. The subjects underwent a sciatic nerve exposure, tracheotomy, and jugular vein cannulation. The animal was then immediately placed in a stereotaxic frame and baseline images were taken. After the baseline images were taken, the animal then received an intraperitoneal injection of guanethidine monosulfate. Two hours after the chemical sympathetic block, the nerve was verified and saline was applied drop wise to the exposed nerve. After reverification of the nerve, 10 images were taken and then a formalin injection was given in the left hind paw. Images were taken for 60 minutes following the formalin injection.

2.5.4 Experiment 4-Guanethidine-Cut

Experiment 4 tested the effects of the chemical sympathetic block and DRRs on blood perfusion. The subjects received the same experimental treatment as subjects in Experiment 3, however, instead of saline, lidocaine was applied to the exposed nerve, and after the allotted time, the nerve was cut. Following the nerve cut, ten images were taken. After the ten images, a formalin injection was given, and 60 minutes of imaging followed the injection.

2.6 Statistical Analysis

Mean blood perfusion for each paw was generated from the laser Doppler imaging software from the regions of interest (ROI). The regions of interest were the left hind paw and the right hind paw. Percent change of blood perfusion was calculated

for standardization purposes and calculated using Equation 1. The baseline average was calculated by averaging the 10 images of the baseline period.

(1) $$\%change = \frac{\left(X - baseline\_average\right)}{baseline\_average} * 100$$

At baseline, both the contralateral and ipsilateral paws in both the non-sympathetic block groups and the sympathetic block groups were not expected to be significantly different in terms of blood perfusion. A repeated measures analysis of variance (ANOVA) was used to assess differences at baseline between the non-sympathetic block groups and the sympathetic block groups. In the non-sympathetic and non-DRR block group, both ipsilateral and contralateral paws were expected to increase in terms of blood perfusion following formalin when compared to baseline. The ipsilateral and contralateral paws at the formalin time period were not expected to be significantly different in terms of blood perfusion. A repeated measure ANOVA with post hoc tests (Tukey's) was used to determine differences between the baseline and formalin time periods, but also to assess differences between both paws at the formalin time period. Additionally, no significant differences were expected between paws during the saline time period or the baseline period. No significant differences were expected in either paw when the respective baseline period was compared to the saline period.

The second hypothesis was that following a sciatic nerve transection, there would be less of an increase on the ipsilateral side compared to the no cut group following formalin. There was no expected change in blood perfusion following formalin on the contralateral side compared to baseline. A repeated-measures ANOVA with post hoc tests (Tukey's) was used to determine differences between the formalin time periods on the ipsilateral and contralateral sides in the cut and no cut groups.

The final hypothesis stated that there would be an increase in blood perfusion bilaterally in the sympathetic block group following formalin. Also, the sympathetic block group with the cut group would have a unilateral increase in blood perfusion to the ipsilateral side following formalin. A repeated-measures ANOVA with post hocs (Tukey's) was used to determine differences between baseline and formalin time periods in the sympathetic block group, but also to determine differences between the ipsilateral and contralateral side at the formalin time period. The analysis would also be used to determine differences between baseline and formalin time periods in the sympathetic block group with the DRR block group

CHAPTER 3

RESULTS

At baseline, both the contralateral and ipsilateral paws in both the non-sympathetic block groups and the sympathetic block groups were not significantly different in terms of blood perfusion At baseline, there was not a significant overall interaction of time, group, or side for mean blood perfusion, $F(9,504)= 0.015$, *ns*, or for percent change, $F(9,504)= 0.166$, *n.s.*

3.1 Formalin injection caused increase of ipsilateral blood perfusion

The first hypothesis stated that there would be an increase in blood perfusion to both ipsilateral and contralateral paws following formalin in the saline-no cut group. A repeated-measures ANOVA showed an overall interaction of time and side for mean perfusion, $F(91,728)= 13.9825$, $p <.0001$, and for percent change, $F(91,910)= 12.319$, $p <.0001$. Post hoc tests for mean perfusion and percent change revealed that there was not a significant difference between the baseline period and formalin time period for the contralateral paw, $p>.05$. There was a significant increase in blood perfusion between the baseline time period and the formalin time period, $p<.0001$ for the ipsilateral paw for both mean perfusion and percent change (Figure 3). The ipsilateral paw was significantly greater than the contralateral paw at the formalin time period in terms of mean blood perfusion and percent change, $p<.0001$. There were no significant

differences between paws during the saline time period or the baseline period, $p>.05$. No significant differences were shown in either paw when the respective baseline period was compared to the saline period, $p>.05$.

3.2 Sciatic nerve transection did not decrease blood perfusion

The second hypothesis was that following a sciatic nerve transection (saline-cut group), there would be less of an increase on the ipsilateral side compared to the no cut group following formalin. A repeated measures ANOVA revealed that there was a significant overall interaction for time, side, and group for mean perfusion, $F(91, 1820)= 2.923$, $p <.0001$, and for percent change, $F(91, 2002)= 1.9523$, $p<.0001$. Post hoc tests (Tukey's) revealed that there were no significant differences in the contralateral paw when compared to baseline for mean perfusion, $p>.05$, or for percent change, $p>.05$ in the cut group. There was a significant increase in blood perfusion for the ipsilateral paw during the formalin time period compared to baseline for both mean perfusion, $p <.001$, and percent change, $p <.001$. There was a significant increase in blood perfusion during the lidocaine-nerve cut time period compared to baseline for the ipsilateral paw for mean perfusion, $p <.001$, and for percent change, $p <.001$. The ipsilateral paw was significantly different than the contralateral paw for the lidocaine-nerve cut time period, $p <.001$, and the formalin time period, $p <.001$, for both mean perfusion and percent change.

3.3 Sympathetic block reduced blood perfusion following formalin

The final hypothesis stated that there would be an increase in blood perfusion bilaterally in the sympathetic block group following formalin. Also, the sympathetic block group with the nerve cut group would have a unilateral increase in blood perfusion to the ipsilateral side following formalin. A repeated-measures ANOVA

revealed an overall interaction for time, group, and side for mean perfusion, $F(91,2548)= 2.211$, $p<.0001$, and for percent change, $F(91,4914)= 1.724$, $p<.0001$. Post hoc tests revealed that there were no significant differences between baseline and formalin time periods in the sympathetic block group for the contralateral paw for mean blood perfusion, , $p>.05$, or for percent change, $p>.05$ (Figure 5). There was a significant increase in blood perfusion during the lidocaine-nerve cut time period compared to baseline for the ipsilateral paw for mean perfusion, $p <.001$, and for percent change, $p <.001$. There was also a significant increase in blood perfusion from baseline to the formalin period for the ipsilateral paw in the sympathetic block group for mean perfusion, $p<.0001$, and percent change, $p<.0001$. The ipsilateral paw showed significantly greater amounts of blood perfusion than the contralateral paw during the formalin time period in the sympathetic block group for mean perfusion, $p<.0001$, and percent change, $p<.0001$. Post hoc tests revealed that there was a significant difference between the formalin time period of the ipsilateral paw compared to baseline for mean perfusion, $p<.001$, and percent change, $p <.001$, indicating that the sympathetic block reduced blood perfusion. There was a main effect for time for mean perfusion, $F(91,2548)= 77.697$, $p<.001$, and for percent change, $F(91,4914)= 40.355$, $p<.001$. Post hoc tests revealed that there was a significant difference between image 18 of the guanethidine period through image 49 of the same period compared to all images of the baseline period for mean perfusion, $p<.01$, indicating that the guanethidine model was effective in blocking sympathetic activity. However, for percent change, only image 49 of the guanethidine period was significantly different from baseline, $p<.05$. There was also an interaction effect for time and group for mean perfusion, $F(91,2548)= 25.815$, $p<.001$, and percent change, $F(91,4914)= 21.082$, $p<.001$. Post hoc analysis revealed

that there was a significant difference between images 39 through image 49 of the guanethidine period for mean perfusion when compared to baseline, $p<.05$ in the guanethidine cut group. Percent change post hoc analysis revealed no significant difference between any images in the guanethidine time period in the cut group compared to baseline, *n.s.* In the no cut group, there was a significant difference between baseline and images 33 through 45 of the guanethidine period for mean perfusion, $p<.05$. For percent change, there was no significant difference between the baseline and guanethidine periods, *n.s.* In the guanethidine-cut group, there was a significant increase in blood perfusion between baseline and formalin time period for the contralateral paw for mean perfusion, $p<.05$, and percent change, $p<.05$ (Figure 6). There was also a significant increase between the lidocaine with nerve cut period, $p<.0001$, and the formalin time period, $p<.0001$, compared to baseline for the contralateral paw for mean perfusion, but not for percent change, $p<.05$. There was a significant increase from the baseline period to the formalin time period for the ipsilateral paw for mean perfusion, $p<.0001$, and for percent change, $p<.0001$. There was also a significant increase between the baseline time period and the nerve cut time period for the ipsilateral paw for mean perfusion, $p<.05$, and percent change, $p<.05$. The ipsilateral paw showed significantly greater amounts of blood perfusion than the contralateral paw during the formalin time period for mean perfusion, $p<.001$, and for percent change, $p<.001$. The sympathetic block group was also compared to the no sympathetic block group, to determine the contribution of the sympathetic nervous system to neurogenic inflammation. A repeated measures ANOVA found no significant overall interaction for time, group, and side for mean perfusion, $F(90,2340)= 1.08$, $p>.05$, or for percent change, $F(91,2366)= 0.048$, $p>.05$.

CHAPTER 4

DISCUSSION

The purpose of the present study was to determine the role of the sympathetic nervous system and DRR on neurogenic inflammation. The present study focused on how these to physiological components effect blood perfusion. It was predicted that following formalin, there would be a bilateral increase in blood perfusion compared to baseline. This hypothesis was not supported. The results indicate only a unilateral increase in blood perfusion to the ipsilateral side. An alternative explanation for this is that due to activation of the sympathetic nervous system, any vasodilatative effects of the DRR would be canceled out by the vasoconstrictive effects of the sympathetic nervous system. However, the sympathetic block group did not induce an increase in blood perfusion in the contralateral paw, indicating that the explanation for the no sympathetic block group would not fully explain the results. However, Lin, Zou, Fang, and Willis (2003) found that sympathectomized animals showed a reduction in DRR-mediated flare responses by capsaicin injection, indicating that the sympathetic nervous system and nociceptive DRR play intricate roles and may influence each other during neurogenic inflammation.

The second hypothesis stated that there would be less of an increase in blood perfusion in the ipsilateral paw in response to formalin injection. This hypothesis was supported for mean perfusion, indicating that there was less of increase following

formalin in the DRR block group. This indicates that potentially DRRs may not have been transmitted to the ipsilateral side, ultimately preventing the additional release of substance P and CGRP. However, there was an increase of blood perfusion in the ipsilateral paw following formalin. This indicates that local axonal reflex could play a larger role in neurogenic inflammation than previously anticipated. Local axonal reflex occurs when the nociceptive action potentials are transmitted to neighboring branches of the axon, causing additional release of neurogenic neurotransmitters, such as substance P and CGRP. Additionally, there was an increase in blood perfusion following the lidocaine application of the sciatic nerve transection, indicating that the blood perfusion could have been affected by this manipulation.

The final hypothesis stated that there would be an increase bilaterally in the sympathetic block group. This hypothesis was not supported. There was only a unilateral increase in blood perfusion following formalin. According to Lin et al.'s (2003) finding that sympathectomy attenuated DRR following capsaicin injection, this finding supports the present results. With an attenuation of DRRs in sympathectomy, there would be an expected reduction in the release of substance P and CGRP, causing no change of blood perfusion to the contralateral side.

The sympathetic block seems to greatly influence changes in blood perfusion. The potential mechanism for this could be explained by the peripheral release of norepinephrine by the sympathetic nervous system. When norepinephrine is released, it may bind to excitatory α_1 adrenoceptors located on the primary afferent terminals, causing depolarization. If this depolarization reaches threshold, an action potential

would generated, causing additional release of inflammatory neurotransmitters. During the sympathetic block, norepinephrine would not be released allowing binding to α_1 adrenoceptors and causing depolarization. This explanation is supported by the findings of Wang et al.(2004). This study found that DRRs were reduced following capsaicin injection with pretreatment of a α_1 antagonist. This experiment also found that DRRs could be restored in sympathectomized rats that were pretreated with a α_1 agonist. Also, α_2 adrenoceptors have also been implicated in the modulation of noxious stimulation in the periphery. Fuchs, Meyer, and Raja (2001) found in normal human skin that α_1–selective agonist and α_2-selective-agonist decreased heat thresholds and increased the subject's pain ratings, indicating that α_2-adrenoceptors also are activated by norepinephrine to cause excitation at the primary afferent terminals.

Additionally, it was expected that following a sciatic nerve transection of the sympathetically blocked group, there would be a unilateral increase to the ipsilateral paw. However, there was a bilateral increase in blood perfusion following formalin injection in the sympathetic and DRR block group. The contralateral side's increase can be explained by loss of tonic control of blood vessels in the periphery due to the sympathetic block. Since Lin et al. (2003) found that DRRs were attenuated with sympathectomy, the increase could be due solely to the sympathetic block. However, the ipsilateral paw was significantly different from the contralateral paw during the formalin time period. This difference could be explained by local axonal reflex.

The sympathetic nervous system's involvement in neurogenic inflammation still remains unclear. Several studies have found that the sympathetic nervous system

causes vasoconstriction on peripheral venules (Lam and Ferrell, 1993). Additionally, the vasoconstrictive nature of the sympathetic nervous system during an inflammatory condition still remains unclear. Lam and Ferrell (1993) found that stimulation of the sympathetic efferents in a normal knee joint caused marked vasoconstriction. However, in a carrageenan-treated knee joint, the vasoconstriction was not as prominent. Also, it seems that the sympathetic nervous system may indirectly contribute to vasodilatation and plasma extravasation. Based on the findings of Fuchs et al. (2001), Wang et al. (2004), and Lin et al.(2003), it seems that through the binding of norepinephrine to α_1 adrenoceptors and α_2 adrenoceptors on the primary afferent terminals, afferent action potentials are propagated, ultimately enhancing DRRs and causing additional release of inflammatory neurotransmitters. Due to the obscure nature of the present literature, future endeavors should be conducted in order to bring clarity to the understanding of the sympathetic nervous system and its involvement in neurogenic inflammation specifically, as well as pain in general. Future research should be conducted to look at the role of the sympathetic nervous system in the presence of inflammatory neurotransmitters, like substance P and CGRP. Also, in order to further elucidate the effects of norepinephine on vascular vessels in the periphery, further research should explore the mechanisms of CGRP and norepinephrine on vasodilatation. By understanding the role of the sympathetic nervous system and DRR in nociceptive processes, we will have a better understanding of the physiological state and ultimately create more effective therapies in treating clinical pain conditions.

APPENDIX A

FIGURES

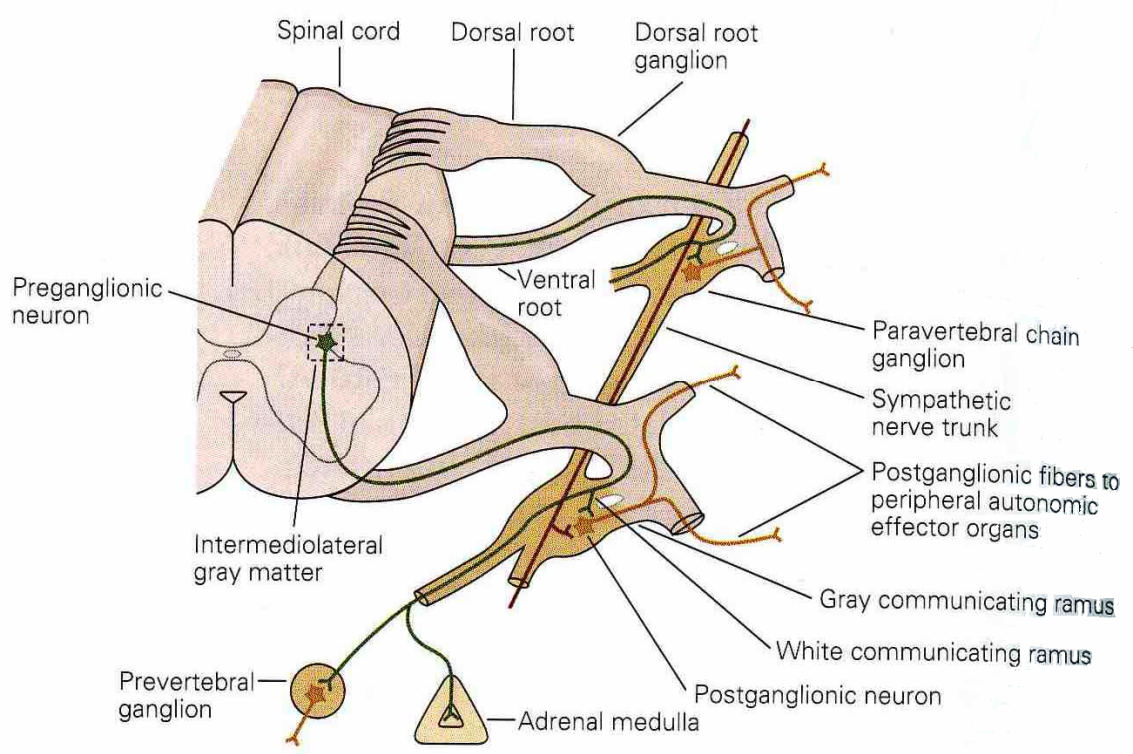

Figure A.1. The sympathetic chain and its connections to the central nervous system. (Kandel et al., 2000)

Sympathetic Block	Sciatic Nerve Transection: No	Sciatic Nerve Transection: Yes
No	n=8	n=8
Yes	n=8	n=8

Figure A.2. Overview of the Experimental Design

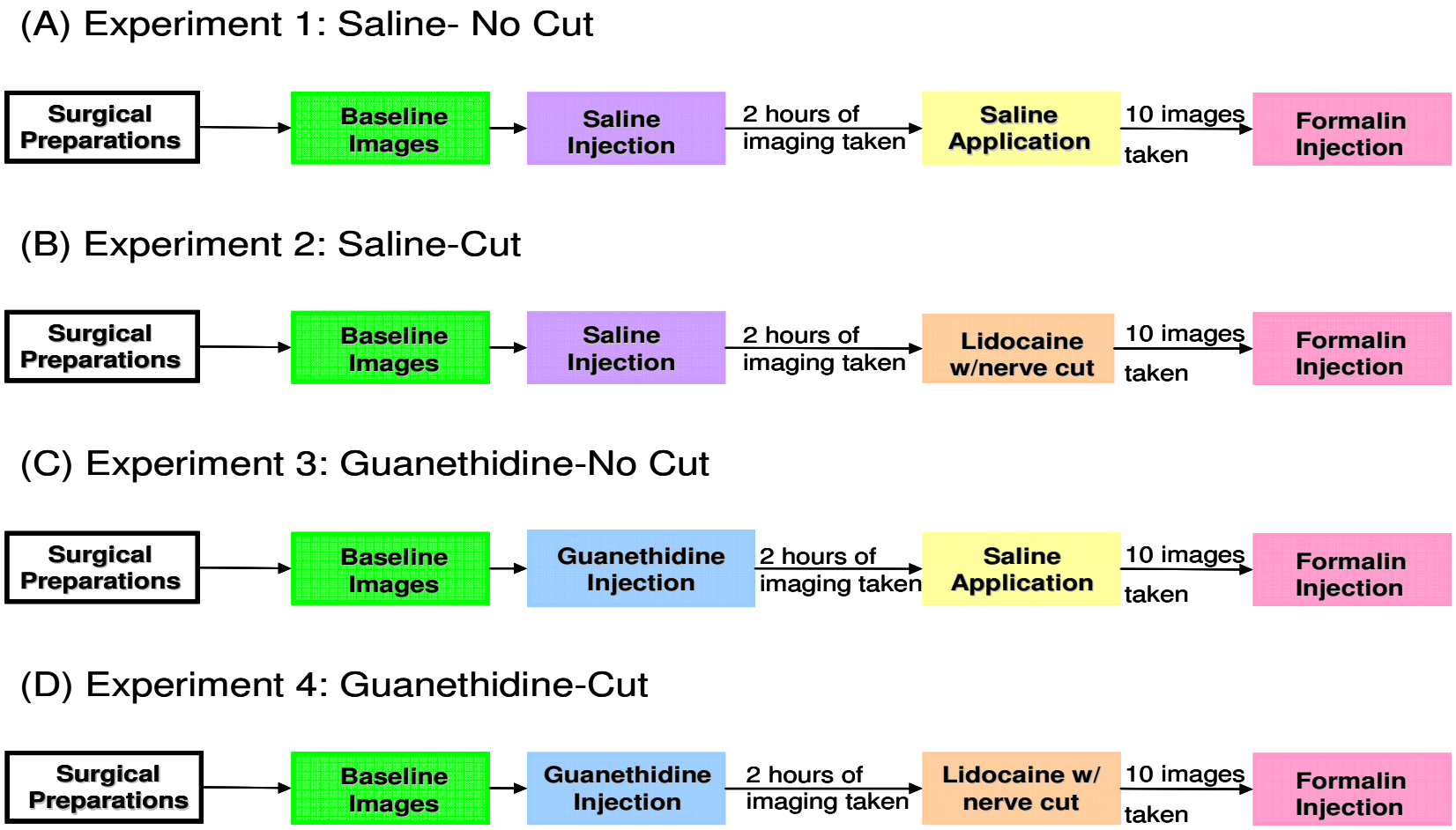

Figure A.3. Description of order of experimental manipulations.

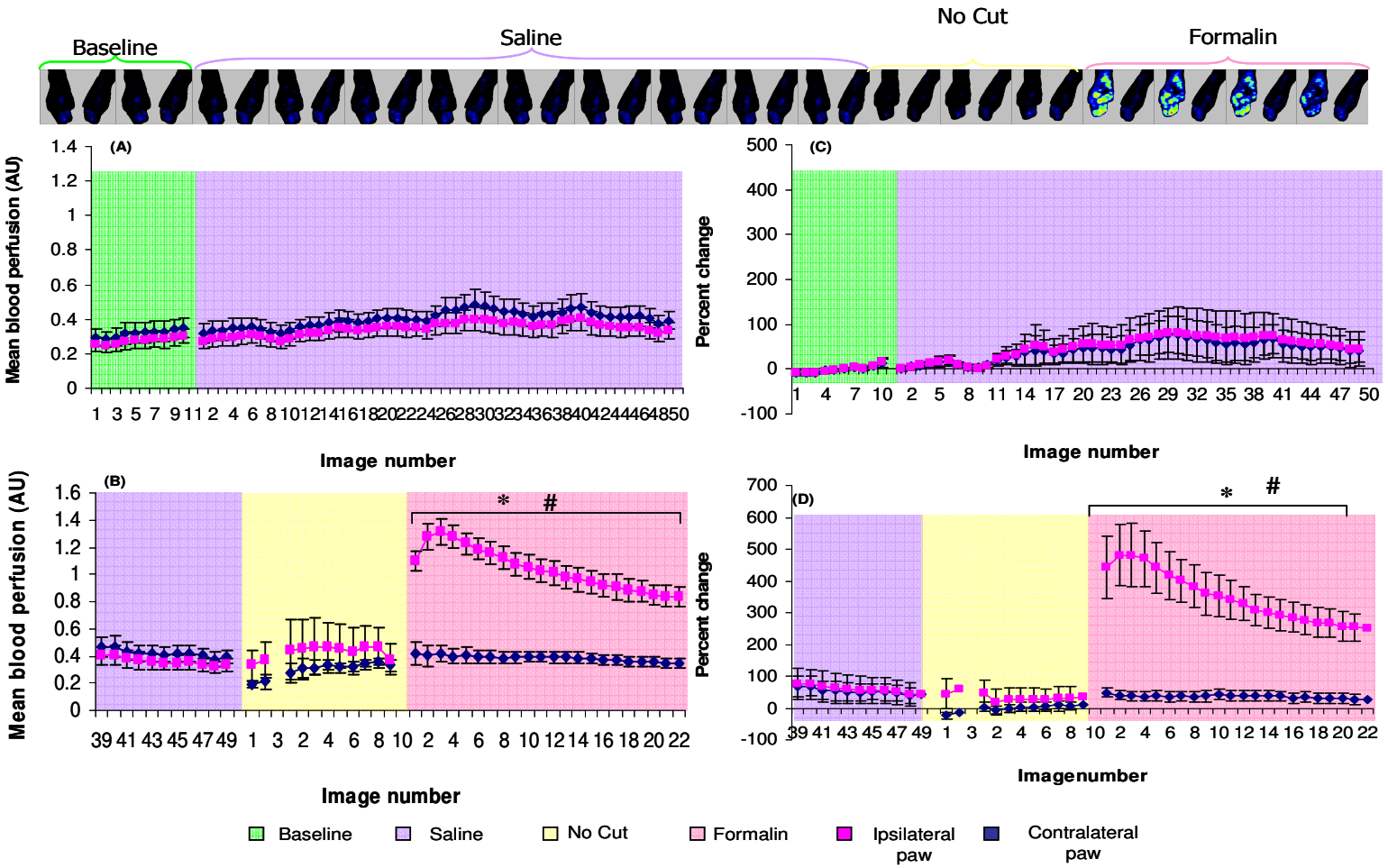

Figure A.4. Mean blood perfusion and percent change for the saline-no cut group (±SEM). (A) Baseline and saline period for mean blood perfusion. (B) Continuation of Saline period, nerve cut period and formalin period for mean blood perfusion. (C) Baseline and saline period for percent change. (D) Continuation of Saline period, nerve cut period and formalin period for percent change. Due to differences in the number of images in each subject, the time periods were standardized, thus each time period was renumbered to begin with image 1. **p*<.05 as compared to baseline for ipsilateral paw, #*p*<.05 as compared to contralateral paw of the same time period.

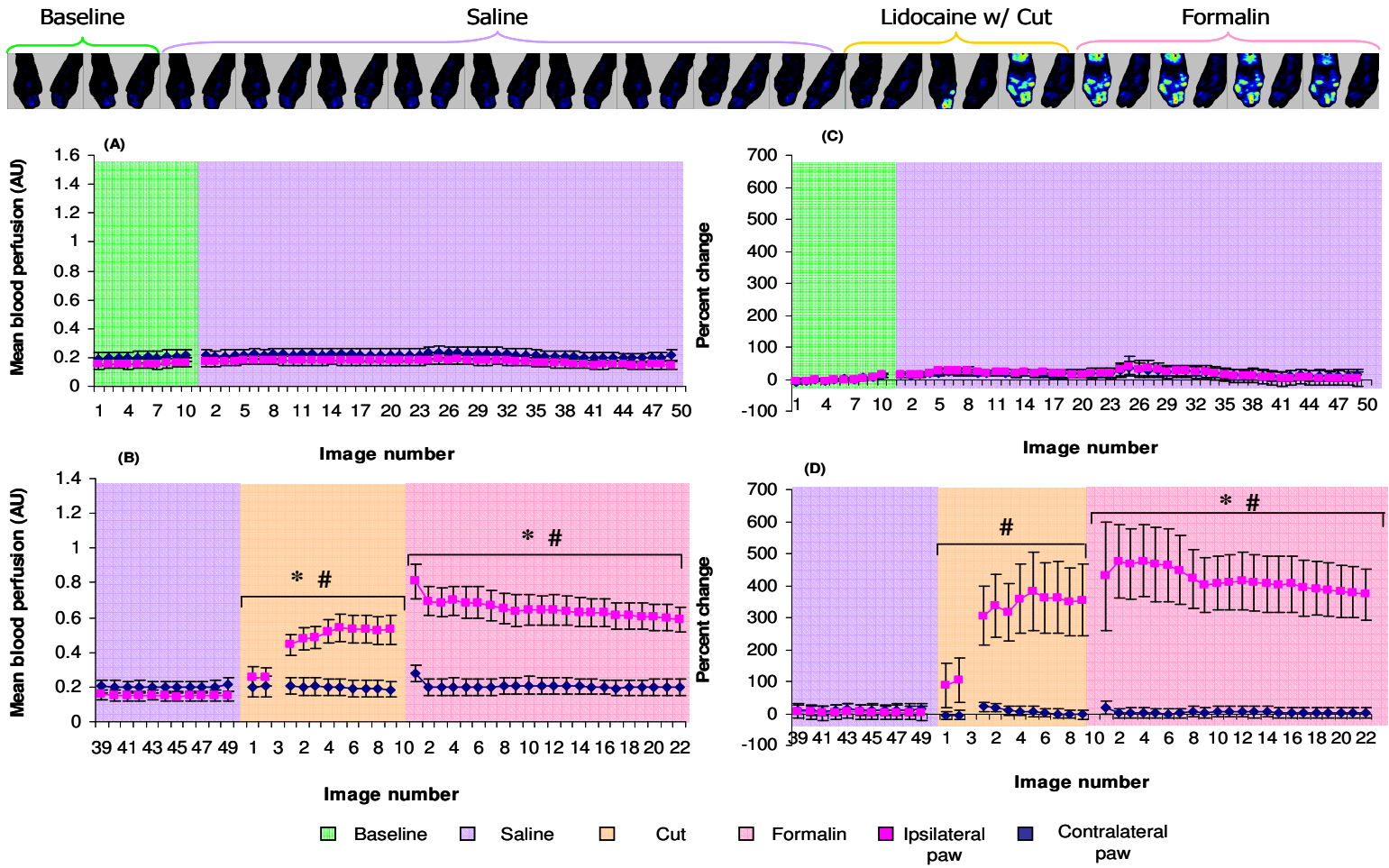

Figure A.5. Mean blood perfusion and percent change for the saline-cut group(±SEM). (A) Baseline and saline period for mean blood perfusion. (B) Continuation of Saline period, nerve cut period and formalin period for mean blood perfusion. (C) Baseline and saline period for percent change. (D) Continuation of Saline period, Nerve cut period and formalin period for percent change. *p<.05 as compared to baseline for ipsilateral paw, #p<.05 as compared to contralateral paw of the same time period.

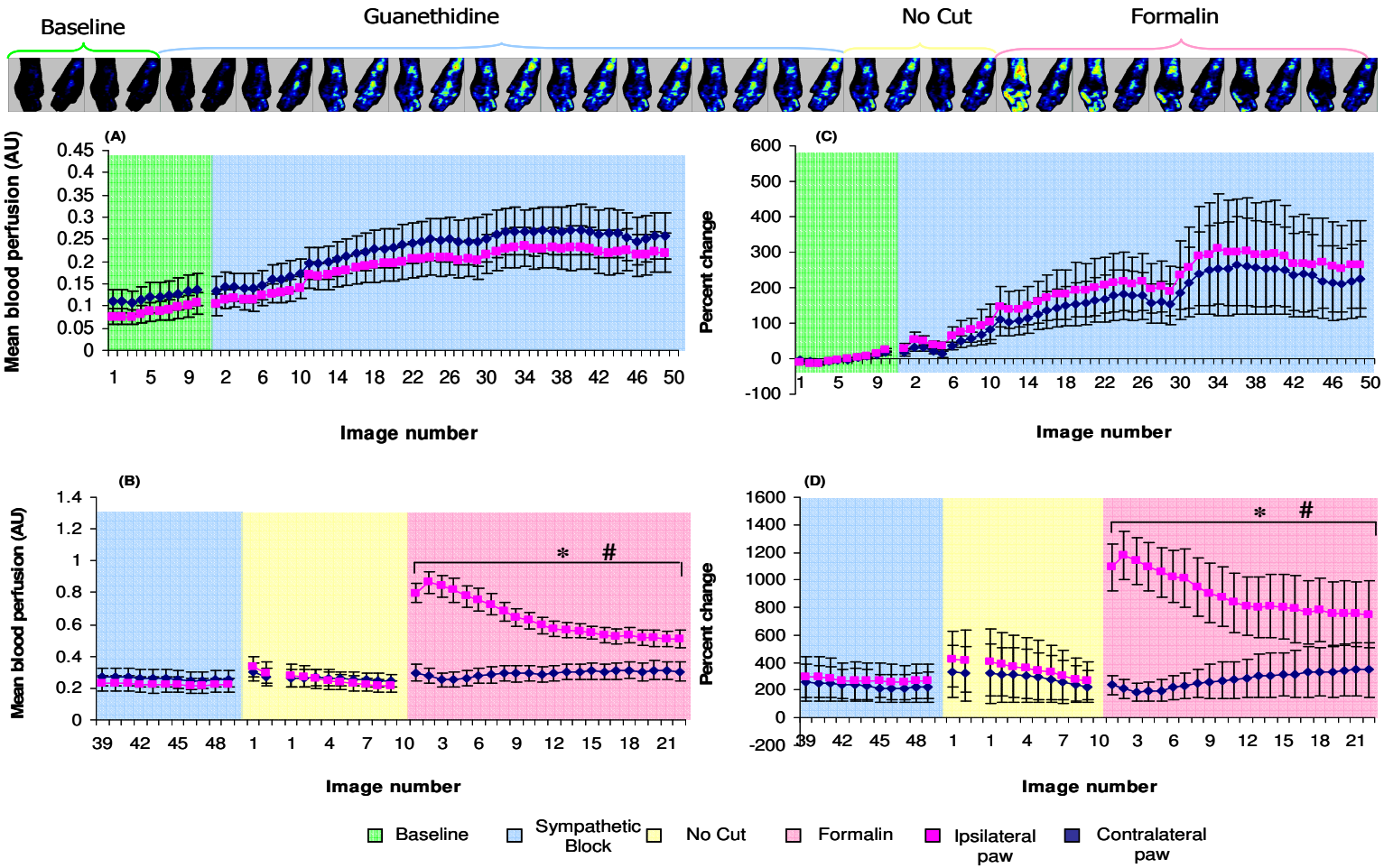

Figure A.6. Mean blood perfusion and percent change for the guanethidine-no cut group (±SEM). (A) Baseline and guanethidine period for mean blood perfusion. (B) Continuation of guanethidine period, DRR block period and formalin period for mean blood perfusion. (C) Baseline and guanethidine period for percent change. (D) Continuation of guanethidine period, DRR block period and formalin period for percent change. *p<.05 as compared to baseline for ipsilateral paw, #p<.05 as compared to contralateral paw of the same time period.

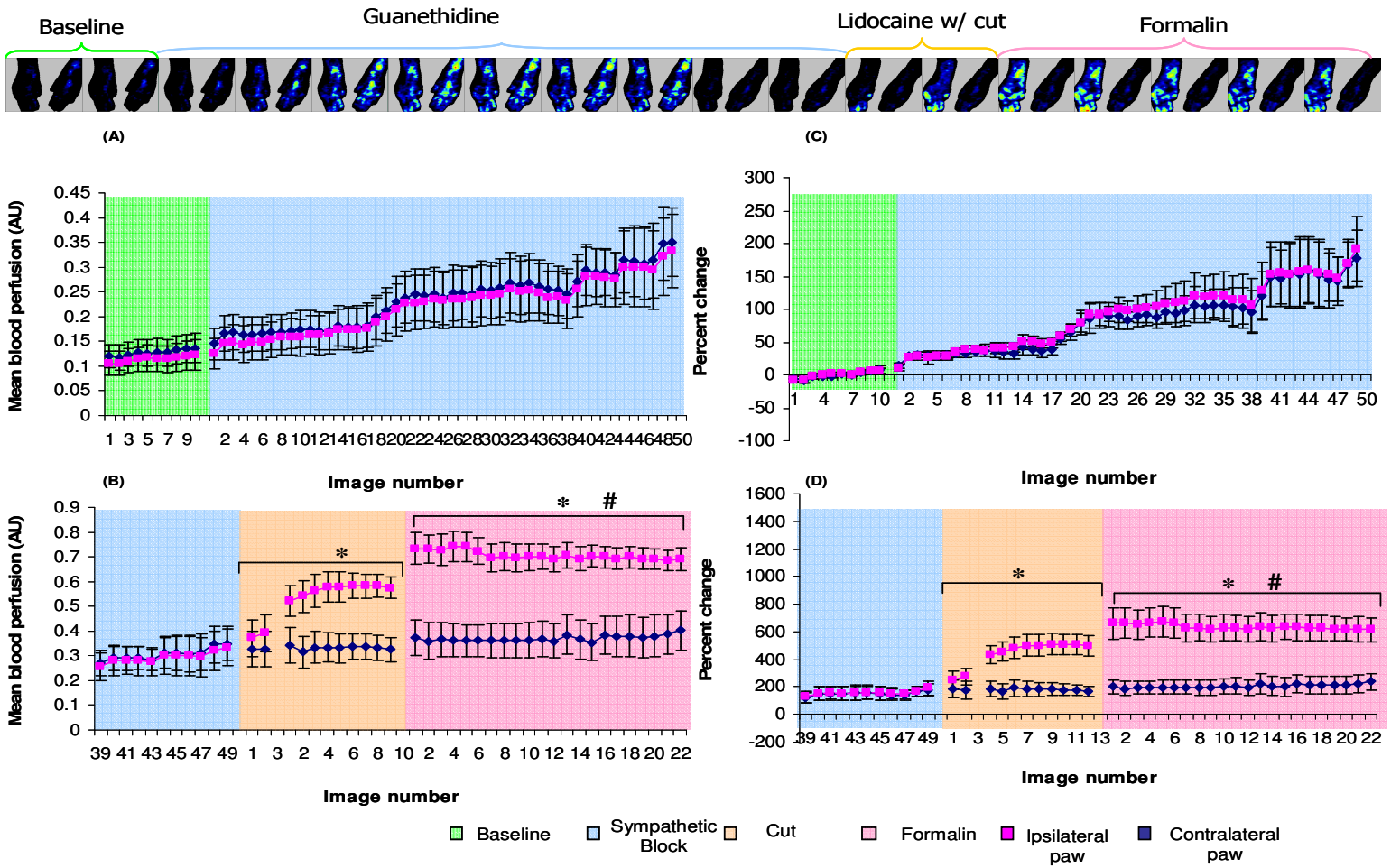

Figure A.7. Mean blood perfusion and percent change the guanethidine-cut group (±SEM). (A) Baseline and guanethidine period for mean blood perfusion. (B) Continuation of guanethidine, nerve cut period and formalin period for mean blood perfusion. (C) Baseline and guanethidine period for percent change. (D) Continuation of guanethidine period, nerve cut period and formalin period for percent change. *p<.05 as compared to baseline for ipsilateral paw, #p<.05 as compared to contralateral paw of the same time period.

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BIOGRAPHICAL INFORMATION

Lara A. Kachlic was born in Tyler, Texas and spent most of her childhood in Lindale, Texas. She graduated from the University of Texas at Arlington with a B.S. in Biology with a minor in Psychology. During her undergraduate career, she had the opportunity to work with Dr. Perry Fuchs. She is currently working towards her PhD in Health Psychology at the University of Texas at Arlington under the mentorship of Dr. Yuan Bo Peng. Her general research interest includes mechanisms of diseased pain states. Her research at UTA has looked at underlying mechanisms of the sympathetic nervous system in pain states.