Fall 2010

An animal model for discogenic low back pain

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AN ANIMAL MODEL FOR DISCOGENIC LOW BACK PAIN

by

Candis Schrelle DuBose

An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the Doctor of
Philosophy degree in Biomedical Engineering
in the Graduate College of
The University of Iowa

December 2010

Thesis Supervisor: Professor Tae-Hong Lim

ABSTRACT

Low back pain is a debilitating condition that afflicts millions of people each year. It is characterized by complex biochemical, morphological, and biomechanical changes. However, most believe low back pain arises due to abnormal mechanical loading, inflammation, and disc degeneration. Several studies have investigated radial back pain, but to date, there is only one in vivo animal model for low back pain. Despite advances in science, the causes of low back pain remain unclear and treatments fail to relieve the pain. To better understand the causative factors of low back pain, a reliable animal model is needed. This study was designed to advance the knowledge of the previous in vivo animal model for low back pain by investigating the effects of shear loading on disc degeneration (for a longer duration of time) and discogenic low back pain (in terms of immunohistochemistry) in hopes developing better treatment strategies for low back pain sufferers and to help elucidate the etiology of low back pain.

Adult male Sprague Dawley rats (n=31) were shear loaded for 4- and 8- weeks. Pain behavioral testing was done prior to and after surgery. After sacrifice, immunohistochemistry was used to detect the presence of pain in the intervertebral discs and the spinal cord. Results of this study indicate that the application of an abnormal shear load gives rise to disc degeneration. Histology revealed that all loaded levels as well as the adjacent levels degenerated due to the shear load. Pain behavior testing revealed that the rats did experience pain, however, when combined with the immunohistochemical results, we were able to exclude the pain as pain stemming from the degenerated discs. Surprisingly, we observed that shear loading caused scoliosis of the thoracolumbar spine.

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CERTIFICATE OF APPROVAL

PH.D. THESIS

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ACKNOWLEDGMENTS

This graduate experience has been a long and challenging venture. Many invaluable lessons regarding patience, hard work, determination, and faith have been shared with me during my time here. There are plenty of people who have worked extremely hard to see me through this process and if it were not for them, I would not have accomplished this goal. I would like to acknowledge my Lord and Savior Jesus Christ for the many mountains He has moved for His will and purpose to be done. The first lady in my life, my daughter Kyra, has been my greatest support and motivation. She has been my Angel and her unconditional love has humbled me and allowed me to understand what is truly important. I am grateful to God for choosing me to guide, protect, and share in the life of such a beautiful spirit. To my husband, mother, father, and brothers (CJ and Sean) thank you is never enough. You have lived this experience along with me and have never left me alone. I am blessed to be a part of such a loving, kind, and supportive family. To my aunt Diane, who believed in the value of education and was there to show me purpose when I was blinded by life. To my sister and friend Tarya Harrell who also took the difficult steps with me and has loved my own as her own, from the bottom of my heart-thank you. Jamie Halvorsen, another angel, has been extremely supportive and instrumental to the completion of this degree. I have had a lot of support from various friends and graduate students that have also helped me through this as well and to them I say thank you. Dr. Tae- Hong Lim has provided me with this excellent opportunity and has seen me through to the end. My work would not be possible if it were not for the invaluable help and guidance from Gail Kurriger, who has been a wonderful mentor and friend. I am indebted to her for all she has done. Dr. James Martin and his entire lab has been tremendously helpful and supportive. Thanks to the Spine Lab-Jaehyun Kim, Dongrim Seol, Byeong Kim, and the faculty and staff in Biomedical and Biochemical Engineering. Thank you to AGEP and GAAN for funding

this endeavor and providing tools for success along the way. Dr. Tonya Peeples, Dr. Tarrell Portman, and all of their staff have been great sources of information and encouragement. All of you are awesome and amazing and I am very grateful and honored to have worked with you all! Thank you very much!

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Adult male Sprague Dawley rats (n=31) were shear loaded for 4- and 8- weeks. Pain behavioral testing was done prior to and after surgery. After sacrifice, immunohistochemistry was used to detect the presence of pain in the intervertebral discs and the spinal cord. Results of this study indicate that the application of an abnormal shear load gives rise to disc degeneration. Histology revealed that all loaded levels as well as the adjacent levels degenerated due to the shear load. Pain behavior testing revealed that the rats did experience pain, however, when combined with the immunohistochemical results, we were able to exclude the pain as pain stemming from the degenerated discs. Surprisingly, we observed that shear loading caused scoliosis of the thoracolumbar spine.

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CHAPTER 1

INTRODUCTION

1.1 Overview

1.1.1. Incidence and Prevalence of Low Back Pain

Low back pain is the second most common reason for doctor's visits and one of the most common reasons for missed days at work [1]. Its causes remain unclear, however, it is believed to be caused by degenerative disc disease (DDD) and/ instability. Regardless of the cause, the effects of low back pain are pervasive and costly. To better understand its effects on society as a whole, we turn to epidemiology. Epidemiology is the branch of science that studies the incidence, distribution, and control of a disease in a population [2]. As is dictated in the definition of epidemiology, both incidence and prevalence are included in studies of this type. Incidence determines the rate at which healthy people develop a disease over time. Prevalence tells us how many people in the population have symptoms of the disease at a particular time point. Ultimately, the information garnered from epidemiological studies help health care workers as well as researchers to control the disease and to determine its long-term consequences to society as a whole. The following paragraphs describe the epidemiology of low back pain.

It has been reported that over 80% of the population will experience low back pain at some point in their lives [3]. The lifetime incidence of chronic back pain has been shown to vary depending on the study population throughout the years. For example, a

1969 cross-sectional study of 692 women aged 15-72 found a lifetime incidence of 48.8% whereas a 1983 study using 1221 men found lifetime prevalence to be 69% [4, 5]. In Canada, an 18.7% incidence rate was reported over the course of a year. Among those incidences, 17.2% were mild cases, 1% were intense cases, and 0.4% were disabling cases of low back pain [6]. According to the National Health Institute Survey, in people up to 65 years of age, musculoskeletal impairments were the most prevalent impairment [3]. In particular, back and spine impairments were the most frequently reported subcategory of musculoskeletal impairments with nearly 52% reporting. The prevalence of chronic pain has been shown to rise beginning at age 25, to peak around age 55-64, and then to decrease. In 1988, 7-14% of the population reported having back pain at sometime over the course of a year, while point prevalence ranged from 4-7% [7]. Lifetime prevalence for chronic back pain was found to be 13.8% [7]. The point prevalence ranges from 13% to 30% [3]. More women report experiencing low back impairments than men do [3]. Differences according to race also exist with more whites than blacks reporting pain experiences. The level of education and socioeconomic status also shows interesting differences. The rate of prevalence is inversely related to educational level. Those with less education report pain experiences more so than those with higher levels of education with the less educated group being 50% higher than the best educated group [7]. Those who work in nursing or lumber and building occupations report the highest prevalences. Female nurses have 16% prevalence while the male lumber and building material worker reports 23.9% [8]. Regional differences in prevalence have been reported as well. People living in the west have a higher prevalence of low back pain while those in the northeast have the lowest prevalence. In

North Carolina, a study investigating the prevalence of back pain was conducted in 1992 [9]. Fourteen years later, that same study was repeated and they found that prevalence rates had significantly increased by 162%.

It is evident that low back pain is a major challenge for all concerned. It's important to understand the population of people affected as well as the factors that could contribute to the incidence of back pain. According to more recent reports, low back pain prevalence is on the rise [9]. The disease affects those with lower educational levels, physically demanding jobs, and women disproportionately. Understanding the toll low back pain takes on society will serve best in developing new treatment strategies and help to understand better ways to protect and maintain the spine.

1.1.2 Recovery from Low Back Pain

Most back pain patients recover fairly quickly with little or no functional loss. Studies show that 60-70% recover within six weeks while 80-90% recover within twelve weeks [3]. When age is taken into consideration, not surprisingly, the young are found to recover more quickly than the old [3]. In contrast to patients whose pain is confined to the lower back, those with sciatica tend to recover more slowly [3]. Recovery rates can be affected by a variety of factors. Workman's compensation negatively affects the recovery rate. Place of injury also affects recovery rates. For example, time spent away from work due to a sprain injury that occurred on duty was found to be four times higher than off duty sprain [3]. When determining the amount of time it would take back pain patients to return to work it was difficult to find consensus as to the main causes. Several factors were considered such as age, work environment, psychosocial influences and

location of the symptoms, but no definitive set of causes could be determined [3]. Patients that have suffered with low back pain six months or more have a less than 50% chance of returning to work [3].

1.2. Socioeconomic Impact

Low back pain is a major debilitating disease that affects 80-85% of the population at some point in their lifetime [1]. It is the second leading cause of neurological disorders and second to the common cold in reason for sick leave in the US [8, 10]. Low back pain is the fifth most expensive musculoskeletal disease in the Netherlands [10]. In the United States alone, low back pain has been estimated to cost upwards of $50 billion per year (in 1991) [10]. Around the world, back pain is quite costly as well. It has been reported that in The Netherlands LBP cost between $367.6 million (in direct costs) and $4.6 (billion in indirect costs). In Sweden, total costs were estimated in 2001 as $2.3 billion while in Australia, costs were as high as $7.6 billion [11]. As we can see, the direct and indirect costs to society are staggeringly high, but this disease costs society in many other ways.

People that suffer from LBP are likely to become less productive not only in activities of daily living, but also on the job. Not only do sufferers not perform at optimum levels, they also cost time and money in sick leave and disability claims. It has been reported that LBP has cost up to 149 million missed work days [12]. Men and women share these lost workdays almost equally. In 1999, 27% of men between the ages of 35-44 had instances in which the patient lost work days due to back pain, whereas 24% of women in the same age group had lost workday cases [8]. These lost workday cases

translated into an average of five and seven actual missed work days for males and females respectively. A study population comprised of working US residents examined the time lost due to decreased productivity over the course of two weeks [13]. It was found that back pain sufferers lost 3.9 hours per worker per week. The same study also found that those with back pain were absent the most hours per week when compared with other conditions such as headache, arthritis, and all other pain. So how much money does missed work days translate into? In 1995, the estimated worth of the lost work days totaled over $14billion and in 2002, this number jumped to $19.8billion. There is currently no way to quantify how low back pain affects the everyday chores associated with maintaining a home, children, and spouses. It is reasonable to assume that the time lost being an active member of the family far outweighs any monetary value that could be associated with it.

1.3. Causes and Treatment of Low Back Pain

Low back pain is caused by a variety of factors and its exact etiology is not well understood. It is believed that abnormal mechanical stresses, inflammation, and disc degeneration are the three major contributors to LBP symptoms [14-16]. Abnormal mechanical stresses can be experienced during heavy lifting and bending. During heavy lifting and bending, the spine and its surrounding musculature must help distribute the load. If the load is too heavy, then the spinal muscles must adjust to compensate the load causing the contraction of muscles that would not normally be active. This type of loading, therefore, would result in the uneven distribution of muscles forces in the spinal column. This shift in muscle force distribution creates the condition of instability.

Inflammation is also believed to cause low back pain. Inflammation occurs following injury or chemical irritation as a reparative response. It is known that inflammation leads to the release of inflammatory cytokines which act to enhance the degradation of the matrix of the intervertebral disc and/or affect nociceptive nerve endings. Although pro-inflammatory cytokines are present in the spine in the absence of injury, in injured tissues their levels have been shown to be elevated [17-20]. Strong correlations have been found between disc degeneration and inflammation although, it is unclear whether or not disc degeneration occurs before or after the inflammatory event. More research is necessary to determine the exact role that inflammation plays in causing low back pain.

Disc degeneration, another cause of low back pain, is thought to be a natural occurrence of the spine but can also result from injury and loading events. The spine begins to degenerate within the second decade of life thereby making it difficult to distinguish between the natural aging process and some injurious events. However, it is clear that altered disc mechanics as a result of changes in the disc structure can lead to instability and ultimately pain as the body attempts to compensate for these changes.

There are several risk factors that have been positively associated with low back pain. Among the many are smoking, obesity, level of physical activity, work activity, and vibration. Amongst those who have experienced low back pain within their lifetimes, more smokers than non smokers have been shown to experience LBP more frequently and more severely [21]. In a study performed in the late 70s among a population of 1221 US men, surveys revealed that 68-79% of men who had experienced LBP were smokers before the time of the interview [4]. Lifting has also been shown to be a risk factor. In a

study conducted in the Netherlands (n=861), it was shown that lifting of 25kg or more accompanying flexion at a minimum of 60 degrees was significantly associated with the presence of low back pain [22]. Vibration exposure has also been linked to LBP [23-25]. Occupational whole body vibration has been shown to elicit increased low back pain in tractor drivers as well as in those that frequently drive motorcycles, handle heavy construction material, or drive buses [4, 26]. It has been suggested that due to the excessive mechanical stresses imparted on the spine in obesity, that this may be a risk factor for back pain. One study has found that obese patients have a more forward tilting pelvis than control patients, showing biomechanical differences in movement in obese patients [27]. However, obesity has been both positively and negatively associated with low back pain (which may be attributable to small sample population) and is considered to be a weak positive predictor for low back pain [28].

Psychosocial work issues and psychological distress have also been implicated in low back pain. Emotional distresses, dissatisfaction with the employer, poor appraisal from the employer are all factors that have been thought to play a role in maintaining or prolonging pain symptoms [3, 29]. But no strong evidence has been found that suggests that low back pain is dependent on psychosocial issues. Socioeconomic status may influence the chronicity of low back pain. People with lower educational levels, those who work hourly jobs, and those with lower incomes may not have sufficient access to physician care. Lack of care may exacerbate pain symptoms and create further damage. Psychological issues such as stress, anxiety, and depression have been shown to have an association to the occurrence of low back pain. The same author also asserts that these factors to occur secondary to low back pain but again, this does not validate

psychological factors as having a great influence on the occurrence or length of low back pain [29].

Clinically, there are certain conditions of the spine that are considered causes of low back pain. Spinal stenosis, disc herniation, spondylolisthesis, scoliosis, and degenerative disk disease are conditions that cause low back pain and will be discussed in brief. Spinal stenosis is the condition in which the spinal canal is narrowed, compressing the spinal cord and resulting in pressure on the nerves causing pain in the legs [30]. Injury or the desiccation of the disc can cause the loss of its elasticity leading to tears in the annulus fibrosus. Disc herniation is the condition in which disc material (nucleus pulposus) is displaced out of the center of the disc, through the torn annulus, entering into the spinal canal [31]. The disc material in the spinal canal can compress the spinal nerve roots causing numbness or tingling of the legs and pain. This condition is most commonly seen in the lower lumbar spine. Spondylolisthesis occurs when the vertebra in the lumbar spine slips forward and onto the vertebra below it [32]. It can be caused by a birth defect, degenerative disc disease, or may be the result of a stress fracture. Patients with this condition may have no symptoms or considerable pain [32]. They may develop lordosis (swayback) or kyphosis (hunchback) due to the upper spine falling onto the lower spine. Common symptoms include, muscle tightness, low back pain, and stiffness. Another clinical problem of the spine is scoliosis. Scoliosis is the sideways curvature of the spine and usually develops during late childhood and early adolescence [33]. This curvature causes the patient to have uneven shoulders and hips as well as possible low back pain. Degenerative disc disease, one of the most common causes of low back pain, results in the degeneration of the intervertebral disc [34]. The intervertebral disc can be

thought of as the spine's shock absorber to the loads and twisting movements that it experiences. With aging, the disc becomes less hydrated and develops cracks and tears. The distance between the adjacent vertebrae becomes smaller creating instability. In an attempt to correct the instability, bone spurs may develop. These spurs can protrude in the spinal canal, again compressing nerves roots, resulting in low back pain. Patients with degenerative disc disease may or may not have back pain and could have trouble bending, twisting, or reaching up. Symptoms are not usually continuous but rather they are sporadic and may get better over time.

Low back pain is treated in a variety of ways which reflects the vast causes of low back pain. There are two types of treatment; conservative or surgical. Generally, conservative treatment is attempted before surgery is recommended. Conservative treatments include exercise, bed rest, spinal manipulation, analgesics, and muscle relaxants. In cases where conservative treatment fails and severe pain persists, surgical intervention may be necessary. Spinal fusion is one surgical option that is used when there is motion between two adjacent vertebrae. Doctors use cages, rods, and metal screws to fuse the two troublesome levels. Disc replacement is a relatively new technology that serves as an option to fusion. It involves removal of the IVD and replacement of that structure with an artificial IVD joint. Discectomy (removal of the painful portion of the disc) is generally used in patients with herniated discs. Finally, laminectomy removes all or part of the vertebral arch to relieve the pressure on the nerve roots. The aforementioned treatments sometimes relieve the pain while at other times the pain persists. The variability of treatment success rates illustrates the importance of determining the causes of discogenic low back pain.

1.4. Problem Formulation

The functions of the spine are to support the loads of the head, neck, and trunk; to maintain flexibility; and to protect the spinal cord. It is intricately organized to perform these tasks containing elements that resist loading (annulus fibrosus), provide a natural shock absorbency (nucleus pulposus), and provide stability (facet joints, back muscles, ligaments). Back pain may arise when the function of these structures are compromised. For example, with disc herniation, the nucleus pulposus leaks out of its space and into the canal putting pressure on nerves and causing considerable pain in the back and due to the interaction with the nerves, pain can be felt down the leg. This type of back pain is called radial back pain. It essentially radiates from the source (the lower back) to another area (the leg). There have been several investigations focusing on the basis of this type of pain and what type of biochemical and morphological changes occur due to radial back pain [35-40]. This has lead to better characterization of radial pain and will ultimately lead to better treatment options. However, discogenic back pain, pain thought to arise from the IVD itself, has received much less attention.

Discogenic low back pain is the major symptom of degenerative disc disease (DDD) and is thought to arise from abnormal mechanical loading and/or instability [14-16]. Instability can be caused by a variety of factors, however, its main causative factor is thought to be disc degeneration. With disc degeneration, the structures that comprise the IVD change biochemically and morphologically such that the mechanical properties of the disc are adversely affected. The disc will no longer provide the shock absorbency

it once did, the annulus will bulge and lose its ability to resist tensile loading, and the endplate will develop bony projections that impede nutritional supply. The disc, at this stage, will not distribute the loading on the spine evenly as before, causing a change in the stability of the segments of the spine. The muscles, which help to stabilize the spine, will be forced into unnatural positions as a result of this change in stability. In an attempt to restabilize the spine, unusual muscle forces, resulting from unusual positioning, will act on the spine perpetuating the cycle of instability and degeneration.

The stability of the spine depends on the correct distribution of the forces that the spine experiences. Gravitational and muscle forces act on the spine in an axial compressive load, therefore it is a common and natural load that the spine is continuously subjected to. This axial compressive force, therefore, can be considered a normal force. Most investigators have accepted the theory that the normal compressive load is an axial loading on the spine. However, while Patwardhan and colleagues agreed that the compressive load is a normal loading, they disagree on the path that the compressive force follows. They postulated that this loading acted along a different path termed a follower load path. They ran experiments to test this theory and found that the large physiological loads experienced by the spine could be supported without compromise of flexibility if it followed a path along the curvature of the spine. It follows from this theory, that muscles would play a large role in "guiding" the forces along this path. Therefore, the abnormal force would be experienced when this path is disturbed, particularly when the muscle forces are not acting along the path in the correct manner. One such abnormal force is the shear force that is generated by the action of the muscles

against the spine. Abnormal forces can initiate degeneration through the redistribution of loads as well as introduce the condition of instability by the same means.

DDD involves discogenic pain that is thought to arise from disc degeneration and/or mechanical loading. Disc degeneration, as we have seen, can be the source of considerable pain. It may be exacerbated by inflammation and brought forth through abnormal spinal loading. Many studies have focused on radial pain, but due to the lack of studies regarding discogenic back pain, the origins of discogenic pain remain unclear. It is for these reasons that a viable animal model investigating disc degeneration and low back pain are needed.

1.5. The Purpose of the Study

As previously reviewed, low back pain is a disease state that most people have had experience with. Various treatment modalities have been attempted to alleviate this problem but to no avail. Although low back pain is very well known, its causes still remains unclear. Low back pain is thought to arise from abnormal mechanical loading, degeneration, mechanical instability, or a combination of these. Many studies have focused on radial low back pain and its effects whereas discogenic pain has been studied less frequently. More information is needed to clarify its origins in order to alleviate discogenic low back pain problem and develop better treatment methods. In this study, we hypothesize that abnormal mechanical loading could give rise to disc degeneration and discogenic low back pain. The aims of this study are to investigate the effects of shear loading on disc degeneration and discogenic pain using an in-vivo rat model.

The forthcoming chapter will focus on the clinical anatomy of low back pain as well as review the literature regarding discogenic low back pain. In particular, chapter two will discuss spine anatomy, intervertebral disc degeneration and the mechanisms of low back pain. Discussions regarding the clinical aspects of low discogenic low back pain, inflammation, and the effects of compressive loading will follow. The chapter will end with in-vivo animal models for discogenic pain and the experimental design of the study. Chapter three will focus on the materials and methods used in the study. The selection of animals and the details of the behavioral and immunohistochemical testing will be discussed in this section. Chapter four discusses the histological, behavioral, and immunohistochemical results generated from the study while chapter five discusses the meaning of our findings. Chapter six details the conclusions garnered as well as the possible directions for future study. Surprising findings regarding the effects of the shear load application are revealed in the Appendix.

CHAPTER 2

CLINICAL ANATOMY OF LOW BACK PAIN AND A REVIEW OF THE LITERATURE ON DISCOGENIC LOW BACK PAIN

2.1. Clinical Anatomy of the Spine

The lumbar spine is a complex structure that resembles a long slender column in the shape of an S [Figure 1]. It functions to 1) protect the spinal cord; 2) to provide flexibility to the upper body; and 3) distribute the loads experienced by the head, neck and trunk [41]. The spine (vertebral column) is comprised of fibrocartilaginous intervertebral discs sandwiched between the vertebrae. At birth, there are 33 vertebral levels, however, during skeletal maturation, five bones fuse to form the sacrum and four fuse to form the coccyx. Therefore, the adult spine contains 24 vertebral levels. During infancy, only two of the four characteristic curvatures of the spine can be seen. The thoracic and pelvic curvatures allow the baby's spine to curve in the shape of a C. After three years of development, walking and crawling help to form the cervical and lumbar curvatures. In all, there are four curvatures in the adult spine which form the characteristic S shaped spine seen in the sagittal plane. The forward curvature in the cervical and lumbar region is termed lordosis and the backward curves seen in the thoracic and sacral regions are termed kyphosis. These curvatures exist to allow humans to be able to walk on two feet because it prevents the trunk of the body from leaning forward and allows the head to be balanced over the body's center of gravity with the eyes straight forward [42].

There are three regions of the spine namely, cervical, thoracic, and lumbar. These regions allow for left and right lateral bending, axial rotation, and flexion and extension. The cervical spine allows for head movements such as nodding yes and no. The seven cervical vertebrae (C1-C7) are distinctly different from the other regions of the spine. They are the smallest and the lightest and articulate with the occipital condyle of the skull. C1 and C2 are called the atlas (allows the head to nod yes) and the axis (allows the head to nod no). The axis is the first vertebrae to exhibit a spinous process. C3-C6 resembles typical vertebrae with the exception of the transverse foramina and bifid spinous process. C7, also called the vertebra prominens, has a long and prominent spinous process that forms the characteristic bump on the base of the neck. Twelve thoracic vertebrae (T1-T12) serve as attachment points for the twelve ribs. Their spinous processes are long and point sharply downward and their vertebral bodies are more massive than the cervical vertebrae, but not as large as the lumbar vertebrae. Another distinctive feature of the thoracic region is that they contain costal facets for the attachment of the ribs. The lumbar spine contains five thick bodied vertebrae and is especially resistant to twisting. This is due to the medially facing superior process and the lateral inferior process [42].

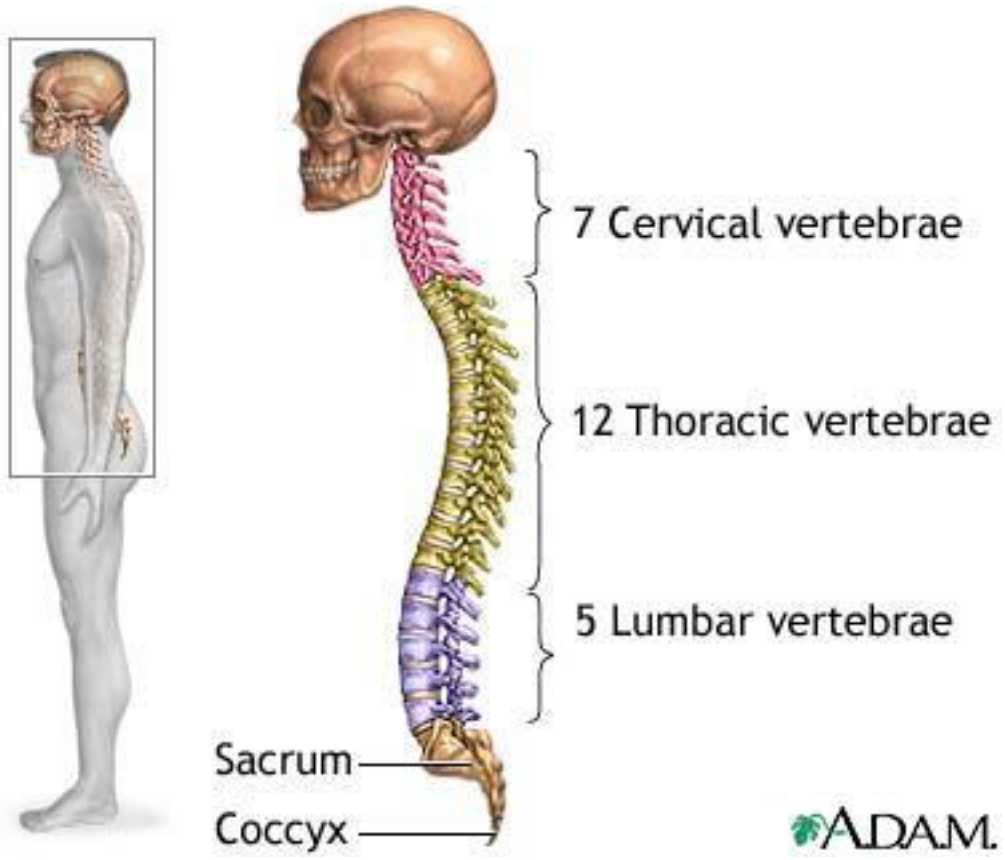

Figure 1: The Human Spine. Illustrates the three regions of the spine-cervical, thoracic, and lumbar.

2.1.1 The Lumbar Spine

The lumbar spine is the focus of many studies because it is the area in which low back pain is experienced. The smallest unit of the lumbar spine that is biomechanically comparable to the entire spine is the motion segment [41]. The motion segment consists of an intervertebral disc between two vertebral bodies bound together by connective ligaments. The vertebral bodies can be thought of a block of bone attached to a ring. The block (vertebral body) consists of a thin cortical shell of bone wrapped around cancellous bone. The ring is called the neural arch and contains transverse, articular, and spinous processes. The spine contains five ligaments: the anterior longitudinal ligament, posterior longitudinal ligament, capsular ligament, intertransverse ligament, and the ligamentum flava.

2.1.2. The Intervertebral Disc

The intervertebral disc (IVD) is composed of three structures: the nucleus pulposus, annulus fibrosis, and the cartilaginous endplate [Figure 2]. The nucleus pulposus (NP) an avascular viscoelastic structure that functions to provide cushion to the compressive, shear, and torsional loadings the spine experiences during normal activities of daily living. It is comprised surrounded by the fibrocartilaginous annulus fibrosis and has a gelatinous core. At birth, the nucleus pulposus has a shiny translucent appearance that changes to a more fibrocartilaginous core with age. Notochordal cells reside in the NP at birth and during early childhood. As we age, however, these cells change to more chondrocytic like cells. The matrix within the core is responsible for the mechanical properties of the disc and is comprised of proteoglycans, collagen (types II, VI, IX, and

XI), elastin, and water (70-90%) [43, 44]. Proteoglycans help to maintain the osmotic pressure in the disc by binding to the water molecules. Collagen fibrils resist tensile loading and serves as a base for attachment of the disc to the bone. The NP is rather large comprising over 50% of the cross-sectional area of the IVD. The spine is subjected to up to 1000N during walking and standing [45]. The individual motion segments experience loads up to five times as much loading at 5000N [46]. With loads this high, optimal functioning of the nucleus pulposus becomes very important for stability.

The annulus fibrosis (AF) surrounds the nucleus pulposus as the outermost portion of the IVD. This structure is composed of laminar concentric bands made up of fibrous annular fibers. Lamellae are individual parallel layers of collagen fibers that attach to the inferior and superior vertebral bodies [47, 48]. The bands are arranged into inner, middle, and outer layers with each layer having alternating orientations and are bound by elastin fibers in between. The fibers are arranged at 30º angles relative to the disc plane and 120º relative to any adjacent band [41]. The AF has fibrocartilaginous cells in the outer portion and fibrochondrocytic cells in the inner portion. These cells are of an ellipsoidal shape and due to the laminar and fibrocartilaginous composition, are allowed to resist tensile loads during normal joint motions and to supply the disc with nutrients [49, 50]. It is comprised of about 70% water with the other 30% owing to solid matrix [47]. The solid matrix contains the negatively charged proteoglycans, matrix proteins, and collagen types I and II. The proportion of these constituents can vary depending on the region (i.e. inner, middle, and outer) of the AF. For example, one study has shown that with increasing distance from the outer to the inner annulus, water and proteoglycan content increases while collagen content decreases [47].

The annulus fibrosis functions to resist tensile loads and to provide nutrients to the avascular disc. Therefore its permeability properties become important in maintaining a healthy disc. One study that investigated the hydraulic permeability of the human AF, found that it is more permeable in the radial direction, followed by the axial and circumferential directions. There was about 26% and 68% less fluid flow in the axial and circumferential directions respectively, than in the radial directions in normal (non-degenerate) IVDs [51]. The diffusion properties of the AF are important as well. One study showed that fluid diffuses through the vessels in the AF in normal IVD possibly exhibiting its role in nutritional support of the structure [52]. It stands to reason that if permeability characteristics are affected, then diffusion characteristics will be affected as well. In a study to determine the diffusion characteristics of the AF, they showed that diffusion is anisotropic occurring in the direction of the alternating laminar layers [53]. Understanding permeability and diffusion within the AF can help to determine the cause of failure in loading situations as well as the health of the disc.

It has been determined that the AF is inhomogenous in regards to its load distribution. Considering the anisotropic structure of the fibers, it is reasonable to expect that the distribution of loads should not be the same in every direction or portion of the AF. According to a study that investigated the response of the AF to compression loads, the AF distributes loads unevenly [54]. The posterior portions of the AF absorb a larger portion of the external loads applied than the anterior portion. An understanding of how the AF distributes loads is an important step in determining the etiology of disc degeneration and ultimately low back pain [55-58].

The cartilaginous endplates are located at the ends of the vertebral body and is composed mostly of hyaline cartilage but contains an osseous component as well [59, 60]. The cartilage endplate provides nutrients to the IVD and resists loads. It is attached to lamellae of the inner AF, and in early skeletal development, has blood vessels that supply the IVD which originate from the spinal artery. The cartilage is composed of proteoglycans reinforced by collagen (mainly type II) fibrils and chondrocytic cells. The EP is selectively permeable more so in the central regions than in its lateral regions where it is almost completely impermeable. In a study of the transport properties of the EP, it was reported that the partition coefficient was affected by the type of solute and composition of the matrix [60], confirming the importance of the EP in the delivery of nutrients to the IVD. It is the EP that is known to be riddled with Schmorl's nodes (leakage of disc components into the vertebral body due to holes in the EP) which can impede nutritional supply to the IVD.

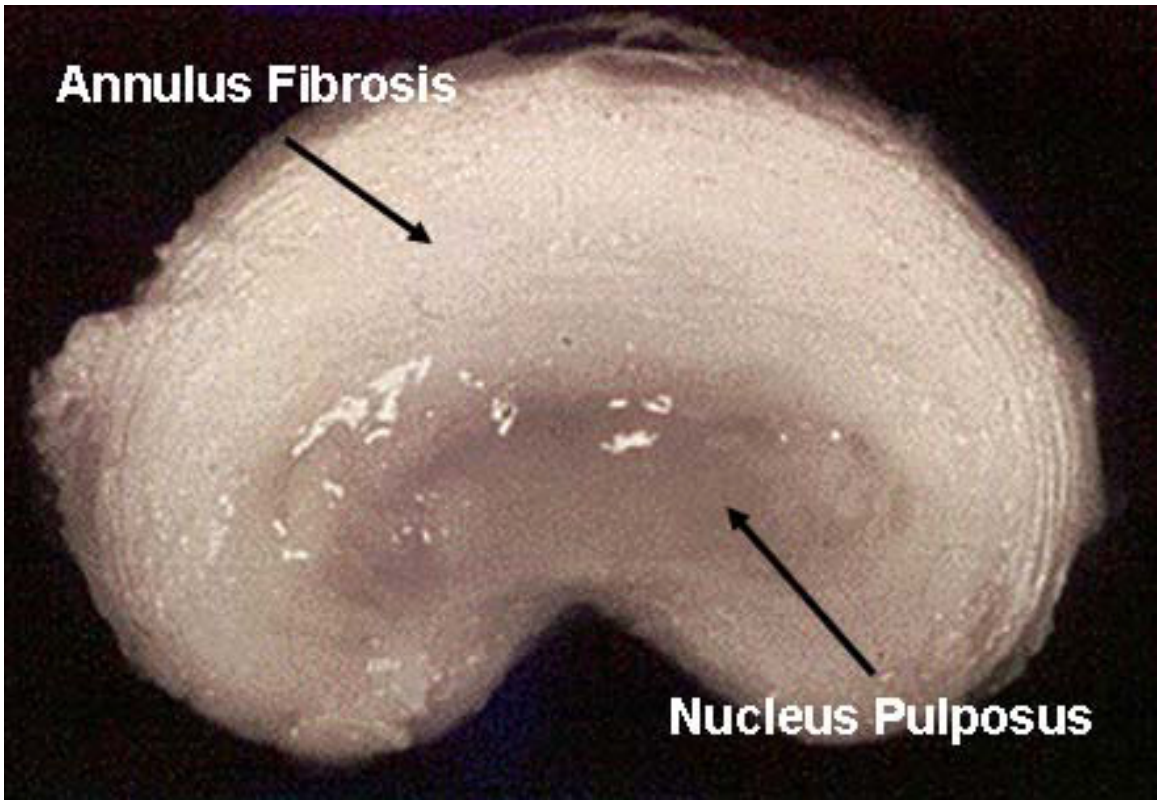

Figure 2: The Intervertebral Disc. Discrete concentric laminar rings of the annulus fibrosus can be seen surrounding the gelatinous nucleus pulposus of the normal IVD.

2.1.3. Ligaments of the Vertebral Column

The motion segment contains five ligaments which can be seen in Figure 3. The anterior longitudinal ligament is attached to the atlas and the anterior portion of all

vertebrae extending down to include a part of the sacrum. It is firmly attached to the edges of the vertebral bodies. The posterior longitudinal ligament covers the dens and runs over the posterior portion of the vertebral bodies down to the coccyx. This ligament is interwoven into the intervertebral disc. The capsular ligaments have fibers that run perpendicular to the plane of the facet joints and are attached beyond the margins of the adjacent articular process. Intertransverse ligaments run between the transverse processes in the thoracic region and are connected with the muscles of the back. The ligamentum flava, known as the yellow fiber, extends from the anterioinferior border of the laminae above to the posteriosuperior border of the laminae below. The ligament is known as the most pure elastic tissue in the body and encompasses the region from the second cervical vertebrae down to the first sacral vertebrae. Interspinous ligaments connect the adjacent spinal bones and their attachments extend from the root to the apex of each process. Supraspinous ligaments originate in the ligamentum nuchae and extend along the tips of the spinous processes down to the sacrum.

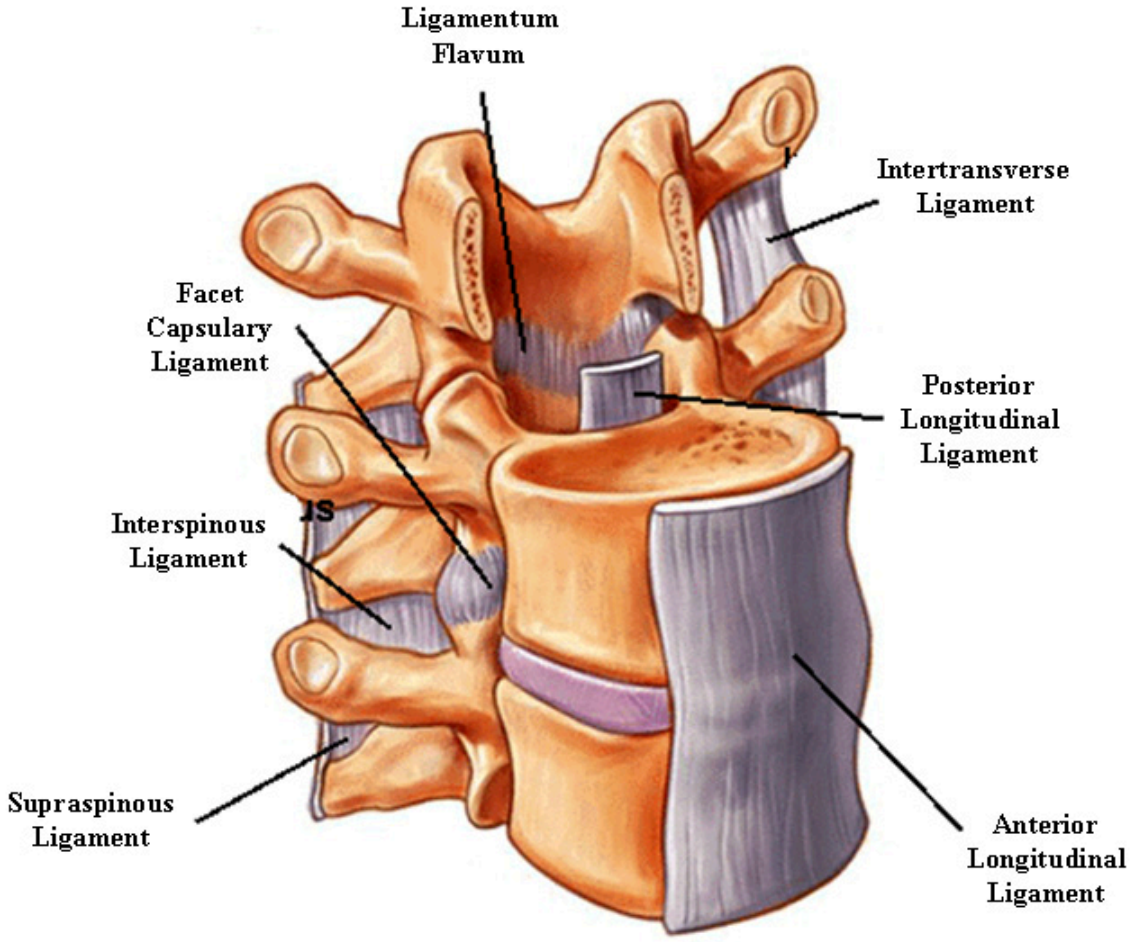

Figure 3: Ligaments of the Lumbar Spine.

2.1.4. IVD Degeneration: Biochemical and Morphological Changes

Disc degeneration is inevitable. It is well known that the disc begins degeneration in the second decade of life. Given that fact, disc degeneration can be a natural consequence of aging. So the question becomes, if the disc is already aging, then how can we define disc degeneration apart from aging? That is a difficult question to answer. Frankly, there is still debate in the scientific community as to what aging looks like in comparison to disc degeneration. Some say that aging should only include changes that occur inevitably and are mainly biochemical in nature while degeneration is a superposition of structural changes in addition to the changes of aging [61]. In an attempt to elucidate a real and working definition of intervertebral disc degeneration, Adams and Roughley, examined changes related to aging as well as changes due to structural failure. They presented a definition of degeneration and a justification of the definition. Adams, et. al., believe that disc degeneration is caused initially by structural failure which leads to an abnormal cell-mediated response [62].

Let us first consider changes in the biochemical and morphological aspects of the IVD as it relates to aging. The IVD is changing rather constantly throughout one's lifetime and depending on factors such as occupation, smoking, weight, and overall health, some intervertebral discs may exhibit more rapid changes at earlier time periods. This makes the task of determining changes related to normal growth and development as opposed to degenerative changes difficult at best. To clarify this issue, studies have been conducted to evaluate both morphological changes and biochemical changes either separately or jointly. The results of the studies of age-related changes in the biochemical compositions of the IVD showed that matrix degradation leads to decreased

proteoglycan content [63]. Proteoglycan content in the NP was found decrease along with the size of the molecule with age [64]. The proportion of aggregated proteoglycans decreases while the proportion of non aggregated proteoglycans increases. The disc volume increases and the inner annulus fibrosis expands due to an increase in fibrocartilage. This expansion increases the distance between the central portions of the disc and the peripheral blood vessels that surround it making diffusion of nutrients more difficult. The water concentration decreases and fissures and cracks can be seen.

One study evaluated the extracellular matrix for changes in newly synthesized aggrecan, newly synthesized types I and II procollagen, and denatured type II collagen [65]. They also graded the morphological changes according to Thompson's scale which is discussed in detail subsequently. Using these tools, they were able to classify changes into three distinct phases: growth, aging and maturation, and degeneration and fibrotic. They found that during the growth phase (0-15 yrs), there was active synthesis of aggrecan and procollagens I and II and increased denaturation of type II collagen. The aging and maturation phase (15-40 yrs) was categorized by a reduction in synthesis of type II procollagen and denatured type II collagen. The degeneration and fibrotic phase (40-80 yrs) showed no increase in type II procollagen or aggrecan synthesis, an increase in denatured type II collagen and a slight increase in type I procollagen.

Regarding the structural changes in relation to aging, Boos, et al. conducted a large study to classify the age-related changes in lumbar intervertebral discs using morphology only [66]. Though this study recognizes that its classification system still needs work, it provides a starting point for classifying changes related to aging. They used 44 cadavers that ranged in age from fetal to elderly for a total of 180 complete

lumbar segment slices. Using these spines and 30 surgical specimens, they were able to establish a system classifying the age related changes in the intervertebral disc summarized in Tables 1-2. Boos, et al were able to provide the morphological evidence of the effects of decreased blood vessels in the endplate which many authors believe is the site of initiation of the deterioration of the IVD. Most of the changes attributed to aging are also occurring in the degenerative disc and variations can be seen in the morphology of the spine across the age groups. For example, Boos, et al. found samples in the elderly to correspond with the same morphological changes in the young. Attempts are still being made to clarify this issue.

GROUP	Nucleus Pulposus	Annulus Fibrosus	Endplate
Fetal (n=9)	Notochordal cells	No abnormalities	Slightly Irregular
0 to 1 month (n=13)	↑chondrocyte density; slightly mucoid degeneration	No abnormalities	Disorganized cartilage
2 months to 2 years (n=17)	Granular changes;	No abnormalities	↓physiologic vessels; obliterated vessels
3 to 10 years (n=5)	↑cell death, granular changes; chondrocyte density	No abnormalities	↓physiologic vessels; cartilage cracks
11 to 16 years (n=8)	↑ cell death; chondrocyte proliferation	Cleft and radial tear formation	Cartilage cracks; microfractures of subchondral bone

Table 1: Age related changes in Human IVD (fetal to 16 years)

Source: (Boos, et al. "Classification of age related changes in lumbar intervertebral discs." Spine 2002, 27(23), 2631-2644); ↑=increase, ↓= decrease

GROUP	Nucleus Pulposus	Annulus Fibrosus	Endplate
17 to 20 years (n=8)	↓chondrocyte proliferation, cell death, mucoid degeneration	↑clefting and tearing	Similar changes as in previous groups
21 to 30 years (n=32)	↑cell density; decaying cells, mucoid degeneration	Clefts and tears	↑Similar changes as in previous groups
31 to 50 years (n=11)	Cell proliferation; mucoid degeneration	↑ clefts and tears	Structural disorganization
51 to 70 years (n=9)	Huge clones of hypertrophic chondrocytes	Clefts and tears filled with granular changes; neovasularity	Microfractures; bone sclerosis; scar formation; advanced tissue destruction
Above 70 years (n=38)	No differentiation between IVD structures; scar like tissue formation	No differentiation between IVD structures	Cartilage disorganization; new bone formation

Table 2: Age related changes in Human IVD (17 years to above 70)

Source: (Boos, et al. "Classification of age related changes in lumbar intervertebral discs." Spine 2002, 27(23), 2631-2644); ↑=increase, ↓= decrease

Endplate fissures are associated with advanced ageing along with irregularities in its morphology [62, 66]. Endplate deterioration has been linked to traumatic injuries to the spine. These injuries can lead to cracks in the endplate which have been shown to disturb the mechanical function of the spine [67]. Several studies have been done to determine the effect of normal and abnormal loading on the EP. One study used finite element methods to determine the effects of mechanical forces on the development of annular tears, nuclear clefts, and endplate fractures. A normal forty-one year old disc-body-disc unit was scanned using axial tomography and was loaded in axial compression, flexion, and extension. It was predicted that the endplate was the weakest structure and was always the origination point of failure within the IVD.

Studies done to evaluate the state of normalcy or degeneration within the IVD have been performed using discography or MRI [68]. Three different types of degeneration within the AF were identified. Rim lesions are vascular defects located at the junction of the endplate and AF. They appear to have been ripped from the vertebral body and can be seen as a result of traumatic injury or degenerative processes elsewhere within the IVD structure [69]. In a study that examined twenty-seven cadaveric spines, rim legions were seen most often in the anterior regions of the disc. Circumferential tears are located on the anterior, posterior, and lateral portions of the AF and are normally seen in conjunction with rim lesions [68]. Vascular ingrowth can be associated with this type of tear as well. The last type of degeneration is the radiating cleft that is normally seen extending from the NP to the outer AF. This type of tear can lead to prolapsed and herniation due to the extrusion of nuclear material. Radiating tears were shown to be

found in the posterior portions of the AF in the lower lumbar spine as compared to other regions [69].

Biochemically, the annulus fibrosus change greatly as degeneration progresses [57]. As the AF degenerates, its area increases, water content decreases which leads to an increase in stiffness properties, granulation increases, and its mechanical properties change due to changes in morphology and biochemistry [54, 57, 58, 70]. Postacchini et.al, conducted a study to investigate the changes in cells and the extracellular matrix in the AF of rats during aging [58]. They found that aging AFs had a transition zone that was larger than younger rats and had fewer cells [58]. The cells that were present had very poorly developed intracellular structures. The cells changed from region to region with the outer regions containing fibroblast like cells and the inner regions containing chondrocyte like cells [57, 58, 70-72]. According to this study, the electron dense material found in extracellular matrix of the older rat AF is not comprised primarily of proteoglycans but of degraded noncollagenous proteins [71].

Since degeneration and aging cause a disruption to the normal distribution of its constituents, the mechanical properties of the AF change [57]. As the AF degenerates, water content decreases which leads to an increase in stiffness properties and granulation increases [57, 58, 69, 70]. The increase in the negatively charged proteoglycans causes an increase in the repulsive forces between the fixed charges which in turn causes an increase in the osmotic pressure of the structure. Increases in osmotic pressure cause an increase in expansion stress and in internal pre-stress [57]. The tensile properties of the degenerate AF in various regions were studied to determine the changes in mechanical properties (Poisson's ratio, stress, strain energy density, and elastic modulus) due to

degeneration as compared to the normal AF [58]. The study showed Poisson's ratio to be affected the most by degenerative grade with its values decreasing as degenerative grade increased. The tensile modulus was shown to be higher in the outer regions than in the inner regions and was shown to decrease with increasing degenerative grade.

The nucleus pulposus displays different characteristics when degenerative changes occur. Cellularly, the nucleus pulposus changes from the notochordal cells in fetal development to the chondrocyte-like cells seen in infancy to the more fibrotic cells that can be seen in adults [70-73]. Chondrocyte-like cells enter the nucleus from the endplate and the notochordal cells undergo apoptosis [70, 72]. The cell density decreases and the number of necrotic cells increase [70, 73]. The rich proteoglycan complex in the nucleus pulposus is responsible for the disc's hydrophilic nature and during aging and degeneration; the nucleus depletes its supply of proteoglycans [71]. This depletion causes the disc to reduce its water content and thereby causes a change in disc height and the mechanical function of the disc [70]. The fibrous layers of the annulus fibrosis enter the outer edges of the nucleus pulposus until there is no distinction between the annulus and the nucleus. The disc changes from the opaque gelatinous core to the brown fibrotic core which causes its properties to resemble more solid characteristics than fluid.

For years, many investigators attempted to successfully develop a method of classifying the degenerative changes of the intervertebral disc. Thompson et al devised a grading scheme that classified these changes into five events [74]. The grading system grades the condition of the nucleus, annulus, end-plate, and vertebral body. Figure 4 shows the distinctions between the five grading levels. In grade one degeneration, the nucleus pulposus is described as a bulging gel, the annulus contains discrete lamellas, the

end plate is uniformly thick, and the vertebral body has rounded margins. A disc classified as grade two will have a nucleus that has white fibrous tissue around the periphery, the annulus can be seen with mucinous material in between the lamellae layers, the end plate has irregular thickness and the margins of the vertebral body are pointed. Grade three degenerative discs consist of a nucleus full of consolidated fibrous tissue, extensive mucinous infiltration and loss of clear annular-nucleus demarcation in the annulus, focal defects can be found in the end plate and in the vertebral body, early chondrophytes or osteophytes can be seen at the margins. Grade four degeneration contains a nucleus that has horizontal clefts situated parallel to the endplate, the annulus fibrosus contains focal disruptions, the endplate has fibrocartilage extending from the subchondral bone, irregularity and focal sclerosis in the subchondral bone and the vertebral body can be seen with osteophytes that are less than 2mm. Grade five intervertebral discs have clefts that extend through the nucleus and the annulus. The endplate had diffuse sclerosis, and the osteophytes located in the vertebral body are greater than 2mm.

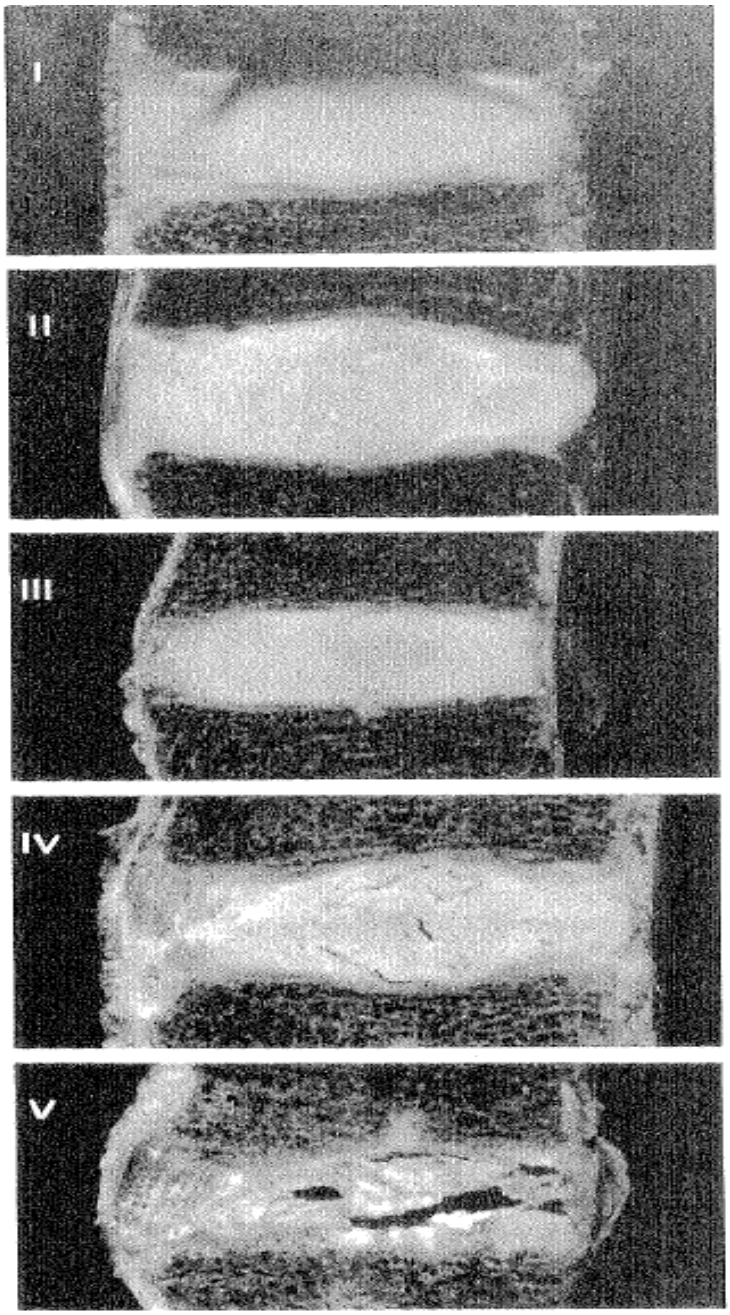

Figure 4: Thompson's Grading Scale

Source: (Thompson, et al. "Preliminary Evaluation of a scheme for Grading the Gross Morphology of the Human Intervertebral Disc." Spine 1990, 15(5), 411-415)

The cartilaginous endplate degenerates in a more distinctive way. The endplate separates from the vertebral body [68]. Cracks can be seen along the ephiseal end. The endplate develops osteophytes and cells from the endplate migrate into the nucleus pulposus. Increases in ossification and calcification can be seen as the endplate degenerate which in turn causes it to become more brittle.

The facet joint degenerates very similarly to joints inflicted with osteoarthritis [75, 76]. Degeneration is marked by cartilage degeneration, subchondral sclerosis, osteophyte formation, and synovial inflammation [77, 78]. The joint becomes stiff, deformed and unstable [36]. Facet joints have been reported to degenerate subsequent to the disc and in doing so, increases the load on the adjacent disc [76-78]. Studies report that the extent of disc degeneration generally correlates to degeneration of the facet joint [76, 77]. Facet joint degeneration was characterized by Moore, et al who used a sheep model with annular rim lesions to study the osteoarthritic changes in the facet joints and to determine whether facet joint degeneration occurred only in levels with injury [76]. Using sheep without injury and sheep with injury at 1-2 months, 4-12 months, and 18-24 months post surgery, they were able to determine that their sheep model is physiologically comparable to the model of facet joint osteoarthritis in humans and that facet joint arthrosis in the sheep occurs subsequent to disc degeneration [76].

2.2. Structural basis of low back pain

2.2.1. Pain Production and Transmission

2.2.1.1 Definition and Characteristics of Pain

Pain, as defined by the International Association of Pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage". It is often the unwanted side effect of some type of injury or disease that most people cannot escape. But pain, outside of prolonged, chronic pain, does serve a purpose. It warns us of injury that is happening currently or of past injuries [79]. Some people may experience pain that is not the result tissue damage but is rather the result of some emotional or psychological experience. Pain is an experience that is unique to each individual. For example, a pinch may be extremely painful to one person, whereas the next person might say that it was only mildly bothersome. Either way, it is an essential part of life as it helps us to protect ourselves from further injury or infection.

2.2.1.2. Types of Pain

Pain can be classified into two types-acute and chronic. Acute pain is pain that begins suddenly and is usually sharp in nature. It can accompany hyperalgesia (an increase in pain elicited by noxious stimuli) and/or allodynia (pain elicited by normally innocuous stimuli) [79]. Acute pain has been considered as a pain that alerts the body, via chemical messengers, of potentially dangerous stimuli that exceed the physiological threshold. It may be severe and long lasting (weeks or months) but usually subsides once

the underlying causes for its presence are resolved. Acute pain never lasts longer than six months. Some examples of this type of pain include broken bones, cuts, and childbirth.

Chronic pain is defined as pain persisting longer than three months. It can arise due to a variety of reasons such as injury or disease, but this type of pain usually outlasts the injury. Some people develop chronic pain in the absence of injury. The effects of chronic pain can be quite costly. Some people develop depression, addiction to medications, or limitations in their daily activities. It is believed that chronic pain serves no physiologic purpose and is not considered normal. Some common examples of chronic pain complaints are backache, headache, and neurological pain.

2.2.1.3. Pain Receptors: Nociceptors

Receptors are free nerve endings located on the ends of peripheral afferent fibers situated in various parts of the body that detect certain stimuli. In the case of pain, these free nerve endings are called nociceptors. Nociceptors respond to tissue damage and the potential of tissue damage. However, activation of these receptors does not constitute pain. Pain only occurs when the brain processes and interprets the nociceptive input.

Nociceptors can reside in many places such as joints, bones, skin, muscles and viscera. The area supplied by the nociceptors is called its receptive field which varies in size according to its location. Nociceptors can respond to many different types of stimuli. For example, some respond to touch, whereas others respond to heat or chemical stimuli. Furthermore, nociceptors can respond to multiple stimuli simultaneously and this type of response is termed polymodal.

There are two different types of nociceptors that respond to pain namely C fibers and A-delta (Aδ). The C fibers are thin (0.4-1.2μm), unmyelinated, slow conducting (0.5-2.0 m/s) and can respond to noxious chemicals, heat or mechanical stimuli. In the skin, the receptive fields of the C fibers range from small (0.2mm diameter) to large (10mm). A-delta fibers are categorized into two types. Type I fibers are activated by noxious high threshold mechanical stimuli as well as weak thermal and chemical stimuli. They are rapidly conducting fibers. After repetitive thermal stimulation, the fibers may become more sensitive to further stimulation causing them to become heat responsive following tissue damage. It is at this point that they become heat responsive and show sustained responses to thermal stimuli of slow latency and long duration. Type II Aδ fibers have a lower response threshold to noxious thermal stimuli. They are less responsive to mechanical stimuli and more responsive to cooling [79].

Nociceptors are unique in that once they have been stimulated they will remain stimulated until the stimulus is removed. Therefore, these pain receptors are non-adapting. As was discussed previously, painful responses to stimuli can be termed either hyperalgesia or allodynia. Two forms of hyperalgesia exist namely primary and secondary. Primary hyperalgesia is a painful response felt at the site of injury. Secondary hyperalgesia is an increase pain response that located at a distance from the initial injury site.

Nociceptors synthesize substances (neurotransmitters) that are released after activation and are involved in the modulation and central transmission of nociceptive information. Some examples of commonly found neurotransmitters include glutamate,

substance P, calcitonin gene related peptide, prostaglandins, and neurotrophins. They are known to exhibit co modulation, co release, and co localization characteristics [79].

2.2.1.4. Pain Transmission

Once injury has been detected via nociceptors, the signal travels up the first order neuron to the dorsal ganglion to the spinal cord. Once there, neurotransmitters are released from the terminal end of the first order afferent fibers onto the secondary neurons located in the dorsal horn of the spinal cord. Depending on the type of stimulation, exact location of the synapse may vary. The dorsal horn, depicted in Figure 3, is sectioned into ten different layers called laminae. In the case of C fibers, they synapse heavily onto lamina II and weakly onto lamina I, V, and X. The Aδ fibers project heavily to lamina I and sparsely onto lamina II and X. Once the neurotransmitters synapse onto the secondary neurons in the dorsal horn, they are then transmitted via the spinothalamic tract to the ventral posterior nucleus of the thalamus (in the case of sharp pain) or to the parafasicular or intralaminar portion of the thalamus (in the case of chronic pain). Third order neurons proceed from the thalamus and then project onto the cerebral cortex where the signal is processed and interpreted as pain [42]. Figure 5 shows the route of pain in pain transmission.

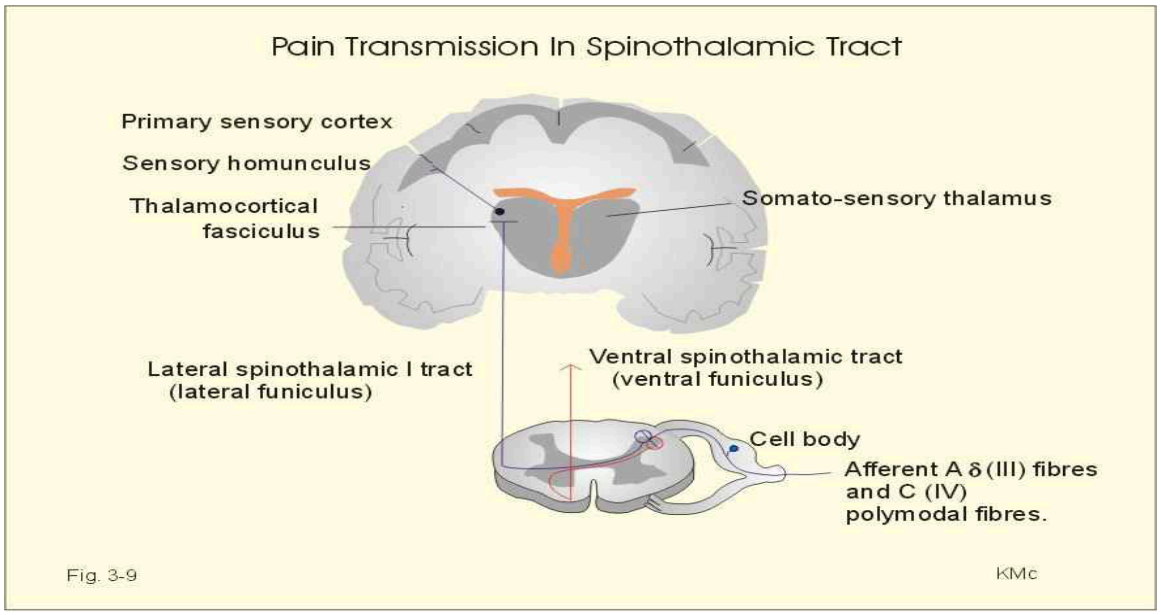

Figure 5: The Pain Pathway. A noxious stimulus stimulates the C- and Aδ fibers. The signal is sent to the spinal cord where it synapses with the secondary neuron and then sends signals to the cortex of the brain.

2.2.2. Innervation of the lumbar spine

The lumbar spine contains five pairs of spinal nerves located proximal and distal to the spinal cord [Figure 6]. The proximal pathway is discussed first. The nerves enter through the intervertebral foramina and have two points of attachment to the spinal cord. As the nerve passes through the intervertebral foramen, it divides to form two branches called a dorsal and ventral root. The dorsal root passes dorsally or toward the back of the spinal cord while the ventral root passes anteriorly or toward the front of the spinal cord. A short distance from the branch point, the dorsal root expands into a dorsal root ganglion which contains the cell bodies of the neurons that carry information to the spinal cord. Then, the dorsal root divides into six or eight rootlets that enter the dorsal horn of the spinal cord. In the case of the ventral root, six to eight rootlets leave the spinal cord to converge and form the ventral root. These dorsal and ventral roots merge and enter the intervertebral foramen to form the sinuvertebral nerve. The sinuvertebral nerve, therefore, is a mixed nerve that carries sensory information to the spinal cord via the dorsal root and motor commands away from the spinal cord via the ventral root. This nerve is the recurrent branch of the ventral ramus (discussed below). It is responsible for the innervation of the posterior portions of the intervertebral disc, the ventral portion of the vertebral column, and the posterior longitudinal ligament [42, 80].

Distal branches of spinal nerves emerge from the intervertebral foramen and divides into a dorsal ramus, ventral ramus, and meningeal branch. The dorsal and ventral rami lead away from the spinal cord. The meningeal branch reenters the vertebral canal and innervates the meninges, vertebrae, and spinal ligaments. The dorsal ramus in particular innervates muscles, joints, skin of the back, as well as the facet joint. The

ventral ramus is larger and innervates the ventral and lateral skin muscles of the trunk. It also gives rise to nerves of the limbs.

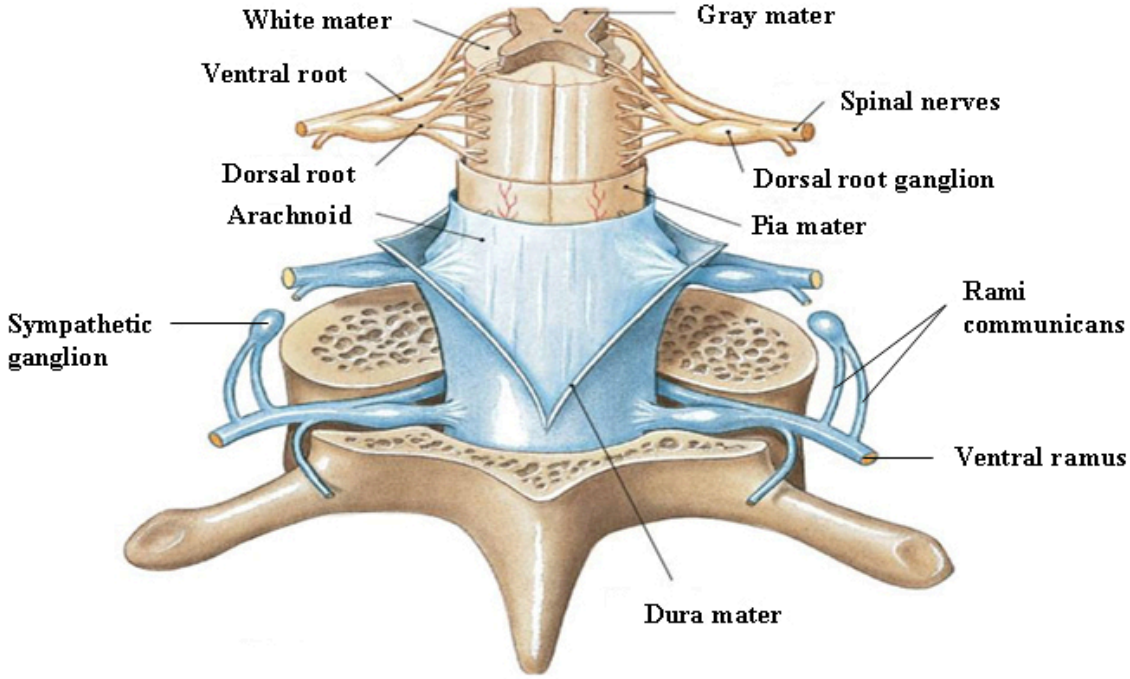

Figure 6: Posterior view of the spinal cord showing spinal nerves, dorsal roots, and the rami communicans.

2.2.3. Neuroanatomical Basis of Low Back Pain

There are many sources of pain in the lumbar spine. Pain can be generated in the free nerve endings of afferent fibers or from damaged or regenerating axons. Initiation of

pain can also occur at higher levels in the spinothalamic tract such as the dorsal horn or the thalamus through neuroplastic changes that help to sensitize higher order neurons [81]. Pain that is generated at the spinal level has been termed central pain. Low back pain can be generated in the dorsal root ganglion or the dorsal roots due to compression or inflammation as well as in the peripheral tissues (i.e. structures of the IVD, muscle). Here we discuss the structures of the spine and how they relate to pain.

Facet Joints: Facet joints are synovial joints and therefore may undergo degenerative changes such as those seen in osteoarthritis or rheumatoid arthritis. The surrounding ligaments and musculature are susceptible to the same degenerative changes as would be seen in surrounding tissues of other synovial joints. Responsible for supporting lumbar extension movements, repetitive loading causes the degeneration of the facet joint due the contact of the superior articular process and the underlying lamina. This joint is heavily innervated with free and encapsulated nerve endings. Some of these free nerve endings may serve as a source of pain [81].

Intervertebral disc: The annulus fibrosus of the IVD has been shown to have within them free nerve endings which may serve as a source of pain. It has also been reported that a consequence of disc degeneration is the growth of nociceptive nerves into the disc itself serving as a source of low back pain [82-85].

Ligaments: The posterior longitudinal ligament has been proven to be densely innervated [86] with nociceptive nerve endings. One study demonstrated that the nerve endings found in the ligaments were immunoreactive for the neuropeptide Substance P as well as calcitonin gene related peptide which are known for their vasodilatory and pain transmitting effects [87].

Vertebral Bodies: The vertebral bodies are bones that contain the same structures of any other bone in the body. The periosteum of the vertebral body is well innervated with nerves originating from the plexus of the anterior and posterior ligaments [88].

2.3. Clinical Aspects of Discogenic Low Back Pain

Clinically, low back pain can be very challenging to treat. In particular, chronic discogenic low back pain with its sporadic nature, makes finding the best solution quite complicated. Fusion of the spine, replacement of the disc, and removal of the painful portions of the disc are current surgical methods employed in the treatment of low back pain. The use of surgical correction does not fully alleviate the problem, but has been deemed helpful. According to a study that evaluated the efficacy of spinal fusion treatment, they found that 63% of the surgical patients showed improvement two years after surgery. While in the case of non-surgical treatment, only 29% of patients report improved conditions [89]. However, as with any surgery, the risk of complications such as infection, device failure, and chronic pain exist. Up to 18% of patients in a statewide study reported post-operative complications. The use of NSAIDs have been proven to be useful in the treatment of uncomplicated low back pain only [90]. Spinal manipulation strategies have also been proven to be effective in the treatment of chronic low back pain. Newer technologies such as disc replacement and introduction of cell replacement therapies have been introduced but have not been definitively associated with better patient outcomes. Discogenic back pain attributed to the degeneration of the disc is a multifactorial problem and may require multiple treatment modalities. The determination of the true causative factors is needed to better treat this condition in the clinical setting.

2.3.1. Inflammation in the IVD

Inflammation is a biological response to injury. It functions to repair and heal the injured site. It is believed that inflammation of the IVD occurs due to disc degeneration. In this theory, as the disc degenerates, the disc loses its proteoglycan content. This is significant because proteoglycans serve to maintain the hydration of the disc. The loss of the small fragments of this aggrecan can generate a signaling cascade which ultimately initiates the production of cytokines. Proteoglycan loss can also affect transport of molecules into and out of the matrix. One of its functions is to serve as a barrier to the transport of larger molecules such as cytokines and growth factors into the disc matrix. Therefore, a loss of this substance potentially provides a gateway for the entry of these potentially harmful proteins [44]. Furthermore, this entire series of catabolic events are thought to be mediated by cytokines [91].

Cytokines are low weight molecular secretory proteins that mediate and regulate inflammation by mediating communication between cells. To accomplish this, they bind to specific membrane receptors which then signal the cell via second messengers to alter their gene expression [92]. There are many different types of cytokines such as growth factors, colony stimulating factors, and proinflammatory cytokines. Pro-inflammatory cytokines, the focus of our attention, work to promote systemic inflammation. Cytokines can interfere with the actions of other cytokines in additive synergistic or inhibitory ways. They are redundant in function meaning that more than one cytokine may perform the same function and are expressed in reaction to a stressor such as a noxious stimulus [93]. They may be found in almost all nucleated cells including chondrocytes, therefore their

presence is normal [94]. Pathological effects are seen when there is impairment of regulatory physiological mechanisms or the inappropriate production of cytokines.

Disc degeneration and herniated discs have been associated with the increased presence of pro-inflammatory cytokines, in particular, interleukin-1 beta (IL-1β) and tumor necrosis factor (TNF-α) [17, 91, 94-98]. IL-1β is produced by the chondrocytes of the annulus fibrosis and acts to accelerate the breakdown of the matrix by stimulating matrix metalloproteinases (MMPs). It is the main pro-inflammatory cytokine in the disc and also acts to inhibit the biosynthesis of proteoglycans by the chondrocytes. Increased levels of IL-1β are seen in degenerated discs and this level increases with increasing degeneration as compared to controls [17]. TNF-α shares similar characteristics of IL-1β. They too are produced by disc cells and inhibit the biosynthesis of proteoglycans. However, TNF-α also upregulates IL-1β, induces collagenase and prostaglandin production, and accelerates the release of matrix components. It may play a role in MMP production. A study that investigated the presence of mRNA of TNF-α in herniated discs showed that the mRNA of TNF-α was expressed more frequently in those discs [98]. It is evident that these cytokines play an important role in disc degeneration even though the initiative factors are still unclear.

In addition, cytokines may also play a significant role in inducing pain. The cytokines may act to sensitize nociceptive neurons in the peripheral afferent nerves and longitudinal ligaments [92]. Sensitization involves increased spontaneous activity and responsiveness to mechanical stimuli. The peripheral afferents then sensitize the central neurons in the spinal cord. This increases the input supraspinally where it is interpreted as pain.

2.3.2 Innervation in the Disc

As previously reviewed, nerves are present in the outer layers of the annulus fibrosus, the endplate, and ligaments. This means that all of these structures can be a source of pain in the IVD. The rabbit annulus fibrosus has both encapsulated and free nerve fibers in the ventral, dorsal, and lateral regions [99, 100]. Facet joint capsules contain many free nerve endings as well [101, 102]. The endplate contains blood vessels and sensory nerve fibers [88]. This is to be expected in normal IVD morphology; however, recent attention has turned to the pathological presence of nerves in the inner annulus and the disc. Studies have shown that nociceptive nerves extend to the inner third of the annulus in the painful IVD and that these nerves carry with them proteins that code for angiogenesis and neovacularization [84, 103]. For example, in a study using cadaveric spines of patients with disc disease, neovascularization of the AF and the NP were shown. Nerves with positive reactivity to the nociceptive neurotransmitter substance P were shown to accompany new blood vessels into the inner third of the annulus fibrosus and in to the nucleus pulposus of the diseased spines [82]. The introduction of nerves into the normally aneural inner annulus and nucleus pulposus implicate the IVD as a viable pain source although the exact mechanisms underlying this type of growth is not well understood.

2.4. Effect of mechanical loads: Previous Studies

The spine is subjected to various loading situations as a result of our activities of daily living. For example, bending moments occur when one reaches down on one side

of the body. Torsional loading may occur when we exercise using weights accompanied with twisting motions. Compressive loading occurs all of the time due to our upright stance and the effect of gravity on the spine. These loading have been known to be in the range of thousands of newtons. Mechanical loading has been long thought to be a major factor in disc degeneration and low back pain although its exact pathomechanism is unknown. Because compressive loading is the most experienced loading situation and the loads on the spine are quite large, scientists have attempted to determine what happens to the IVD as a result of this type of loading. The following section will discuss the investigations of compressive loading in vitro.

2.4.1. Effect of compressive loads: In vitro studies

Stability is a major function of the spine. It is important to determine the characteristics of the stable and unstable spine in order to attempt to determine the causes of its demise. Investigators approach this problem from different perspectives. Studies can focus on the general characteristics of the motion segment as a whole or as it relates to the individual structures of the IVD. In a study that investigated the effects of axial compression on the T9-S1 and T5-L2 levels it was determined that creep rates were smaller in the T10-12 and L1-2 levels. Axial deformations and ventral bulging were shown to increase as one descends the spine. These results demonstrate that the mid thoracic region more stable than the lumbar region and that variations exist in the biomechanical properties within the thoracic and lumbar levels [104]. Another study investigated the creep characteristics of the human lumbar spine during compressive loading. In nine cadaveric samples it was found that in the older population, the creep

rates were greater and the material property values were lower. Lower material property values indicate that the material is becoming less stiff and therefore less able to resist the loads incurred in daily living. The authors concluded that female spines were less stable than males due to increased creep rates in females and that decreased modulus and stiffness were associated with disc degeneration [105].

The role of various structures in load bearing has been investigated as well. MacGlashen et al tested nine L5-S1 cadaveric spines in compression and shear with and without posterior elements. They found that the removal of the posterior elements caused increases in displacements. In other words, the IVD becomes less rigid under load which ultimately causes instability in that segment [106]. Through serial sectioning of the IVD, the load bearing characteristics of the L2-3 and L4-5 motion segment were studied. The authors found that under compression, the IVD is stiffest and the disc is the load bearing structure. The facets were found to play a larger role in stability under posterior shear and axial torque. However, they could not determine the major load bearing structure under combined loadings [107]. The role of the NP in compression was investigated using cadaveric spines. Not surprisingly, it was found that removal of the NP caused serious instability. The most interesting finding was that the job of the NP was to resist lower loads until the AF could take over the load bearing responsibility [108].

Animal models have been used to help determine biomechanical properties of the spine and to determine how well suited animals are for determining human outcomes. Zimmerman used canine motion segments intact, with removal of ligaments, and with removal of posterior elements of levels L2-3 and L5-6 to determine what part these components play in the overall mechanical properties. They found that after removal of

the posterior elements, there was a 15% decrease in the stiffness and a 9% decrease in modulus in the L5-6 level. They were able to determine once again that the posterior elements play a significant role in the mechanical properties of the motion segment. Their results were similar to the mechanical properties found in humans and it was determined that the contribution of the ligaments and posterior elements were similar to humans as well [109]. Race et al. used bovine IVDs to determine its mechanical response to axial compression at different loading rates and hydration levels. This investigation simulated the characteristics of the degenerate IVD via hydration loss. The results lead credence to the viscoelastic properties of the IVD because they showed that loading rate influences the mechanical response of the IVD. Loading rates were in the range of 0.3kPa/sec to 30 MPa/sec. Higher loading rates increased stiffness and decreased the shock absorbency of the IVD [110]. In a study using porcine motion segments, the biomechanical properties as well as water diffusion trends were examined. They too found that higher loading rates increased stiffness properties. It was also determined that water diffusion pathways were disturbed during loading [111]. As we can see, stability depends on a variety of factors. We do know that the posterior elements (facet joints and ligaments) play a major role in maintaining stability. The contributions of the individual structures to the more realistic loading situations have been difficult to elucidate but it is evident that the functioning NP, AF, and EP are necessary to resist loads in everyday living.

The IVD is always changing and as in the case of disc degeneration, these changes can be detrimental. The relationship of the structure and mechanical properties of the IVD in these situations is critical to understand if we are to find ways to cope with

the inevitable disc degeneration. Lumbar cadaveric specimens were tested to determine the effects of degeneration on the distribution of elastic moduli in the lumbar spine [112]. The degree of degeneration was determined radiographically and macroscopically. The indentation test samples were taken at 10X10mm nodal points across a transverse segment of the L3-4 and L4-5 lumbar segments. Distributions were irregular for discs of moderate to severe degeneration with values of 34.4, 48.8, and 69.7 kPa for the NP, anterior AF and posterior AF respectively. The results of this study showed higher elastic modulus values for the degenerated disc as compared to the normal disc. However, disc degeneration was not clearly defined and the use of a polyurethane specimen to estimate disc viscoelastic properties may have skewed the results. Adams, et al conducted a study in which they tested cadaveric spines to elucidate the origin of disc degeneration. They loaded motion segments from T12-L5/S1 to determine 1) the severity of endplate damage in the adjacent level with changes in the distributions of compressive stresses, 2) to determine how discs would respond to cyclic stress and 3) to determine if the disc would be able to equalize stresses before and after endplate damage in flexed and lordotic postures . They concluded that endplate damage leads to increased deformation of this structure causing decreased hydration, decompression of the nucleus, and stress gradient folding of the annulus fibrosis. They believe that these changes will lead to further degeneration [113]. A finite element study was conducted that determine the effect of degeneration on the L3-4 segment. The model showed that mildly degenerated discs showed increases in rotation but that for more severe degeneration cases, the rotation decreased [114]. The literature reveals that compressive loading is not

always helpful to the spine. Its effects on the biomechanical and structural properties of the spine are detrimental and complex.

2.4.2. Effect of compressive loads: In vivo studies

While studies conducted on excised IVDs can provide invaluable information about the system, it is not indicative of the true loading situation. Ideally, we prefer to study a system that more closely matches the problem in its anatomical location. In other words, it is good to see what effects the body has on the functions of the IVD under compressive loading. The following will discuss the characteristics of the spine under compressive loading in the more realistic situation.

Compressive loading in vivo has been studied by several authors [115-121]. Coil springs were attached to the L3-4 level in twelve dogs. After up to one year, the dogs were sacrificed and their spines examined by radiography, visual inspection for morphology, and immunohistochemically for changes in the composition of the discs. They found that in the compressed discs, the nucleus and inner annulus decreased its proteoglycan content and increased amounts of collagen type I [122]. In another study by the same lab, the authors wanted to determine how compressive forces applied over time affected the proteoglycan and collagen production by IVD cells. Again, they coil loaded L1-2 and L3-4 for a period of 13-27 weeks. For the dogs that remained under compressive loading the longest, in the nucleus, there was a strong correlation between force and force weeks and the decrease in proteoglycan content. Rat tail discs were used to determine whether chronically applied compressive forces caused mechanical property changes. Compression was applied via an Ilizarov-type immobilization apparatus for

eight weeks. Significant decreases in disc thickness were found among the compression loaded group. The compression group also exhibited increases in axial and angular stiffness and decreases in proteoglycan content [123]. Another lab loaded 15 rabbits in compression for 28 days followed by 28 days of no compression. The rabbits in the compression group had increases in fibrous tissue in the nucleus pulposus, disorganized annular structure, and increased proliferation of cartilaginous tissue in the AF with increasing load [121]. These in vivo studies clearly demonstrate that in vivo compressive forces can also induce significant adverse mechanobiological effects.

2.4.3. Effects of Shear Loads: in Vitro Studies

Shear loading in the IVD has not been studied; however investigators have examined the effects of shear on articular cartilage explants and cells. In one study, bovine articular cartilage chondrocytes were subjected to shear stress via continuous laminar fluid flow for 2-24 hours. They examined the cells for changes in the release of nitric oxide and found that there was an increase in nitric oxide release that varied with the duration and magnitude of induced shear. Chondrocytes release nitric oxide in response to adverse chemicals such as IL-1 beta and TNF-alpha. Therefore increased levels of nitric oxide may signal the initiation of degenerative changes [124]. Jin et al used young bovine cartilage explants to study whether cell-matrix deformation induced by shear would stimulate the biosynthesis of chondrocytes in the absence of fluid flow and pressure gradients. Shear deformation was applied using 3% dynamic shear strain amplitude at frequencies between 0.01 and 1.0 Hz. With this loading configuration, synthesis of protein was increased by 50% over controls and a 25% increase in

proteoglycan synthesis was found [125]. The same author investigated the effects of tissue shear deformation and insulin-like growth factor-1 on chondrocyte biosynthesis. In this experiment, cartilage explants from young bovine calves were cultured and subjected to sinusoidal shear strain amplitudes between 0.5 and 6% strain for 24 hours. To test the effect of IGF-1, recombinant IGF-1 was added to the media. Shear alone stimulated protein and proteoglycan synthesis by 30-35% and 20-25% respectively [126]. Another study investigated the effects of fluid shear flow on chondrocyte proliferation. Four to six week old bovine calve articular cartilage was harvested and their chondrocytes were isolated and cultured for testing. The cell monolayers were exposed to 3.5 MPa of shear stress in a parallel plate flow system for 96 hours. They found that the shear stressed cells overgrew the monolayer and concluded that shear stress promotes chondrocyte proliferation [127].

2.4.4. Effect of Shear Loads: In Vivo Study

There is only one study to our knowledge that investigated the effect of shear loads on the spine in vivo. Previously, in our lab, Kim et al [128] investigated the effects of shear loading on disc degeneration using an external shear loading device. Adult male Sprague Dawley rats (n=24) were assigned to four groups: immobilization control (n=7), shear loading for 1week (n=6), 2 weeks (n=6), and 4 weeks (n=5). The device was applied to the L5-6 level and at the appointed time of sacrifice, the spines were harvested for histological analysis. The loaded and adjacent levels were examined. After histological examination the control group showed no degeneration with the exception of two IVDs. All shear loaded animals had degenerated IVDs beginning week one and this

degeneration could be seen at adjacent levels as well. Pain behavior measurements using mechanical withdrawal testing were taken as well. The results indicated that the rat's pain coincided with the onset of degeneration. This study was the first to our knowledge to show that shear loading causes disc degeneration.

2.4.5. Limitations in Previous Studies of Mechanobiology of IVDs

The literature provides invaluable insights into how mechanical loading affects the IVD but there are still questions as to exactly how these changes occur. The studies performed are not without limitations. Identification of limitations helps us to determine the next step in our proposed investigations of mechanical loading and its role in IVD function, stability, and degeneration.

There are several limitations to previous studies. The effects of the compressive forces are the only forces that have been studied although these forces are not the only forces acting on the spine. It is evident that compressive forces are considered normal to the spine due to our upright posture and this is the main reason these forces are studied. Previous results have shown that compressive forces are definitely harmful but still, the exact frequency, duration, and magnitude of loading that causes these effects are unclear. Another limitation of these studies is the usage of cells or explants to draw conclusions about compressive forces. Chondrocytic cell monolayer cultures are known to lose their phenotype and the cells used are predominately of a younger population. Disc degeneration starts in the second decade of life therefore information about the effects of compression on a healthy population may not be indicative of the target population. Another limitation of these studies is that abnormal loading situations have not been

studied extensively. Only one known study (Kim et al) has been able to demonstrate effects of abnormal loading and pain. Although this study proved meaningful, there are still questions as to exactly what type of pain was caused. In other words, was this pain discogenic? More studies are necessary to determine the effects of abnormal loading situations and how it relates to discogenic low back pain.

2.4. 6. Mechanics of Stability through Follower Load

The functions of the spine are to maintain flexibility while supporting the loads of the head, neck and trunk. The spine has been shown to withstand large loadings. 1000N of force is generated by walking or standing [45, 129] and during exercise or heavy lifting, loads can be even larger. The spine is a long slender column of which we know flexibility is maintained by the IVD joints. However, the exact mechanisms as the how the spine withstands these large loads are unclear.

Mechanically, the spine can be thought of as a long, slender, flexible column. Columns are known to fail under compressive loading due to large side deflections (buckling). Buckling is a phenomenon that depends on the elastic modulus and cross-sectional stiffness of the column in question. There is one theory that offers an explanation as to how the spine might be able to support such large loads without failing. Patwardhan et al theorized that in order for the spine to withstand large loading, the resultant force must follow a path that is tangential to the curvature of the spine [46]. In this system, the compressive load vector, which is perpendicular to the mid plane of the disc, must pass through the instantaneous center of rotation (ICR) in the center of the segment [Figure 7]. This configuration reduces the coupled flexion-extension moments,

thereby reducing the shear force. They then demonstrated that using this theory, the spine can withstand up to 1200N of compressive load ex vivo. It was also shown that deviation from the follower load path may induce flexion and extension moments as well as shear forces in the spinal segments.

If the follower load is a viable theory, then what controls the path of the follower load? It is believed that the muscles of the spine may work to control the direction of the resultant force vectors in the spine. The idea is that muscles are selectively activated to maintain the resultant of external loads, spinal forces, and body weight passing through the ICR of each motion segment. Based on the findings of Patwardhan and collegues, we are able to reasonably assert the following postulations:

1) The stability and flexibility of the spine can be maintained via the follower load with adequate back muscle control.
2) A compressive load is a normal load applied to all areas of the spine.
3) Instability arises when the follower load path is not produced for any reason thereby resulting in abnormal bending moments and shear forces.

These postulations were the basis for considering the shear force an abnormal spinal load that should be intimately related to instability.

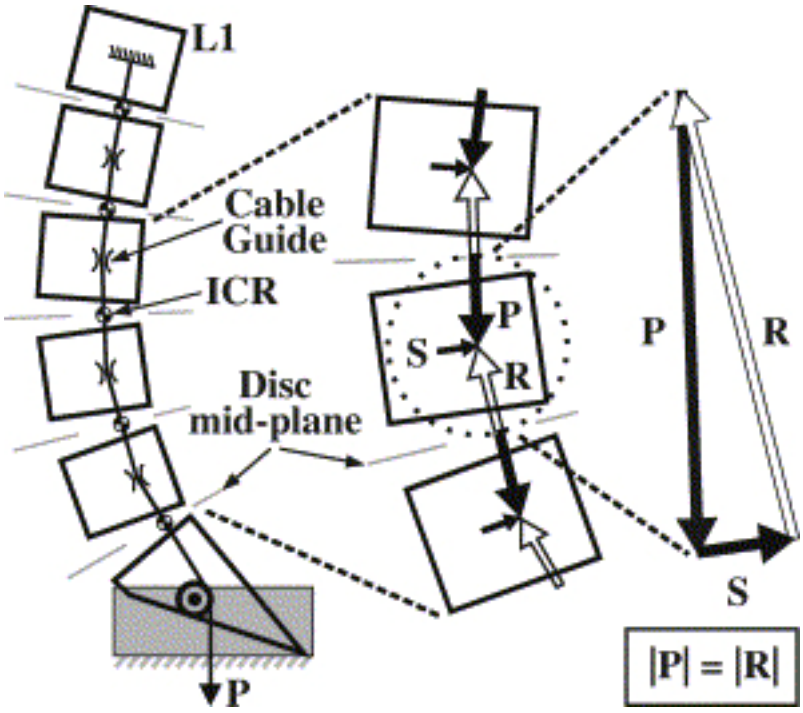

Figure 7: Follower Load Mechanics- This illustrates the experimental setup of Patwardhan et al.'s compressive follower preload. The load vector P passes through the flexion-extension ICR of each segment. Equilibrium of intermediate vertebrae under cable force (P), cable guide shear force(S), and internal reaction (R) are illustrated in the free body diagram.

Source: Patwardhan, et al., "A Follower load increases the load-carrying capacity of the lumbar spine in compression." Spine, 1999. 24(10):pg 1003

2.5. In Vivo Animal Models for Discogenic Pain

To our knowledge, there have been no in vivo animal models of discogenic pain. Instead, studies have focused on creating pain similar to sciatic pain in humans using nerve root ligations. They have also simulated pain emanating from the herniated discs and chemical or mechanical alterations to the dorsal root ganglions. Neuropathic pain can be detected in animals in three ways: autonomy, hyperalgesia, and allodynia. Bennet et al reported on a method to produce pain like sciatica seen in humans. Using 148 male Sprague Dawley rats, this lab exposed the sciatic nerve and ligated the nerve at 1mm increments for a total ligation length of 4-5mm. They used noxious heat testing, behavioral monitoring, and chemogenic testing to determine if the ligation caused pain. Nerve ligation did indeed cause chronic cutaneous hyperalgesia and allodynia resembling the symptoms in man [35]. One lab found that loose mechanical constriction of spinal nerve roots were not ideal for models simulating lumbar radiculopathy. Rather, they suggest that chemical factors from chromic gut ligatures play a role in the development of behavioral changes seen in the rat [39]. The application of tight ligatures produced noxious heat hyperalgesia and mechanical allodynia in the rat. Kim et al found that tight ligatures of the L5 and L6 spinal nerves produced hyperalgesia of five weeks duration and ten weeks of allodynia [40]. The role of inflammatory cytokines and the initiation of radiculopathy in normal and mechanically compressed DRGs were investigated [36]. Local application of exogenous TNF-alpha produced mechanical allodynia that outlasted the duration of application. They also showed that TNF-alpha application to the DRGs exacerbated pain symptoms for about one week. These in vivo animal models effectively

model the sciatic situation but provides little to no insights on the origin of discogenic back pain.

2.6. Experimental Design

2.6.1. Introduction

Many Americans suffer from discogenic low back pain daily. This condition is the second leading cause of doctor's visits in the US and the third leading reason for surgery [3], yet the etiology of low back pain has not yet been well described in the literature. Surgical treatments for low back pain, such as spinal fusion or discectomy have been used, but for some patients these interventions have not been successful.

Several theories have been proposed as to the causes of discogenic pain [116, 130, 131]. It is believed that low back pain is caused due to abnormal mechanical stresses on intervertebral joints and/or disc degeneration with the release of inflammatory cytokines. A viable animal model is needed to clarify the origin of discogenic low back pain and to provide alternative methods of treating people suffering from this condition.

The functions of the spine are to transfer the weights experienced by the head, trunk, and pelvis; to allow motion of the upper body; and to protect the spinal cord. Reports indicate that the spine supports loads in the range of thousands of newtons [8,28]. In order to maintain stability under these conditions, one lab proposes that the resultant forces must be small and act along the curvature of the spine [46]. Since compressive forces are normal forces experienced by the spine, most studies regarding discogenic back pain focus on excessive compressive forces on the spine as the cause of low back pain. The stabilizing features of the spine, however, are the spinal column and

surrounding musculature. When abnormal changes are made to the spine such as damage to the stabilizing structures, it follows, then, that detrimental changes in the loading distribution of the spine might occur. The postulation of a follower load as a normal spinal load may imply that the compressive load is a physiological load in the spine in contrast to a common belief that the compressive load in the spine may be the major mechanical cause of low back problems. When the follower load (presumably a physiological load) is broken, a shear force should occur in the spine either in the sagittal plane or in the coronal plane. Such a shear force may be an abnormal spinal load that may result in the spinal deformity as well as the degenerative changes associated with back pain.

2.6.2. Follower Load: Possible Role of Shear Force on IVD Degeneration and Discogenic Low Back Pain

Discogenic low back pain is thought to arise from two main factors: abnormal mechanical loading and inflammation resulting from disc degeneration. Patwardhan et al theorized that the resultant loads on the spine must follow the follower load path in order to maintain flexibility under large loading. They present this loading pattern as a natural, normal physiological load. If this does exist, then the axial load would be the normal load and the bending moments would be used for spinal motion. Back muscles play a critical part in maintaining this load path. If this is the case, then it is reasonable to assume that the endplates, central portion of the disc, and the subchondral bone region would be subjected to anterio-posterio shear forces resulting from the back muscles. The application of the abnormal shear forces to these areas of the spine could initiate

instability and the biochemical and structural changes (degeneration) that accompanies it. To date, there is only one study that investigates the role of shear forces in disc degeneration and low back pain. From these results, we saw that the application of the abnormal shear force produced disc degeneration and hyperalgesia. However, we have not confirmed whether or not the pain behaviors seen in those experiments resulted from the disc itself (discogenic low back pain).

2.6.3 Pain in the Disc

Tissue damage and the resulting inflammatory/chemical factors are known to elicit low back pain responses. In the periphery, nociceptive nerve endings send information to the spinal cord where the neurons in the spinal cord are sensitized (central sensitization). The pain information is then sent supraspinally to the thalamic region and eventually to the brain where it is interpreted as pain. The disc is subjected to constant stress as we perform activities of daily living. Therefore if the disc is injured, it would be subjected not only to inflammation which can lead to pain, but also to mechanical stresses in conjunction with the release of nociceptive substances. It stands to reason that this type of combination would exacerbate pain and also lead exaggerated responses in peripheral and central sensitization.

Freemont et al and others have reported the growth of nerves and blood vessels into the painful degenerated disc. They have found that the nerves are nociceptive and contain the nociceptive neurotransmitter Substance P [17, 18, 84, 103, 132]. This study will employ the use of immunohistochemistry to detect the presence of inflammatory

cytokines (IL-1beta and TNF-alpha), the nociceptive neurotransmitter Substance P, and the vasoendothelial growth factor (VEG-F) in the subchondral bone, and disc area.

2.6.3.1 Pain Assessment using Secondary Hyperalgesia

Pain is manifested uniquely in each individual. It is subjective and thereby requires that we communicate with others to alert them of our condition. The use of animals in pain studies presents us with the challenge of deciphering whether or not the animal is experiencing pain using traditional methods. Fortunately, there are manifestations of pain in animals that are recognizable to researchers. In pain research, behavioral signs of pain (nocifensive behaviors) are monitored. These behaviors include spontaneous behavior, and responses to noxious or innocuous stimuli. This study uses tests of mechanical hyperalgesia as a measure of pain.

Hyperalgesia has been shown to result from deep tissue at a site distant from the initial site of injury [133, 134]. Studies of spinal nerve injury or disc herniation, show hyperalgesia to mechanical or heat stimuli [40, 135-138]. This is comparable to the symptoms of back pain. For example, back pain patients may experience pain in the back (primary site of injury) as well as pain in the leg (secondary site).

2.6.3.2 Mechanism of Hyperalgesia

The mechanisms of primary hyperalgesia are well known. The injury occurs at a peripheral site which signals the release of nociceptive neurotransmitters. These neurotransmitters act on the free nerve endings of the peripheral afferent neuron resulting in pain at the primary site. The mechanisms of secondary hyperalgesia, however, are not

as clearly understood. It is believed that secondary hyperalgesia is the result of central sensitization of the neurons in the dorsal horn of the spinal cord. Central sensitization produces lower thresholds and spontaneous neuronal firing that manifest as hyperalgesia and allodynia. In this case, the peripheral afferent neuron sends the nociceptive information to the dorsal horn of the spinal cord. There, the primary neurons sensitize the neurons of the dorsal horn which then send the information to the thalamus and ultimately to the brain where it is interpreted as pain emanating from the primary injury site. Central sensitization cannot occur in the absence of primary sensitization. The initiation of central sensitization is thought to arise from the prolonged activation of C fibers. This triggers N-methyl-D-aspartate-dependent calcium entry and post transcriptional changes in signaling pathways [139, 140].

2.6.4. Evidence of Pain in the Spinal Cord

To further confirm the presence of pain, we searched for two proteins. P-creb is in its unphosphorylated state known as creb. It is a protein that has been associated with long-term potentiation, is known to enhance synaptic transmission, and is responsible for the transcription of immediate early genes containing the cAMP response binding element (CRE) sequence in the promoter region. The cascade of activation of creb is as follows. The cell signal arrives at the cell surface which activates receptors and produces a second messenger such as cAMP or Ca^{2+}. This in turn activates a protein kinase (modifies proteins through phosphorylation) which moves to the nucleus and activates CREB protein which binds to the CRE region. This allows the genes to turn on or off. In

the early stages of inflammation, CREB was shown to be increased in the dorsal horn [141]. One of the immediate early genes that is phosphorylated by CREB is c-Fos.

C-Fos has been shown to be a marker of pain [141-143]. It is a proto oncogene whose expression is rapid and transient. Upon inflammatory or painful stimuli, transcriptional activation occurs within minutes with accumulation of its mRNA occurring within 30-40 minutes after stimulation [144]. We believe that this known pain marker and its activation source will be present in the dorsal horn of the spinal cord as a result of chronic pain induced by disc degeneration that has manifested as hyperalgesia. Since projecting neurons are required for the transmission of pain to the brain, we look to these specific areas of the dorsal horn –superficial laminae I, II, III, and IV.

2.6.5. Postulated Mechanism of Discogenic Low Back Pain

Scientists agree that the causes of low back pain are numerous but degenerative disc disease (DDD) is the most widely accepted cause. We postulate that discogenic low back pain arises due to aberrant mechanical stress and/or disc degeneration inflammation. Inherent in this reasoning is that DDD is closely related to instability. We postulate that the cartilage endplate and subchondral bone are the sites of discogenic pain for several reasons. 1) The cartilage endplate (CE) disappears with aging [37] or annular puncture induced disc degeneration (unpublished data). Histological signs of neovascularization and bone cell migration into the central region of the disc are also observed with NP and CE changes. 2) An increase in sensory nerve endings is found in CE and SB obtained from patients during anterior interbody fusion surgery for treating painful DDD by Brown et al. [145, 146], suggesting an increase in blood flow as an attempt to augment

the nutrition of degenerating discs. 3) The presence of CE defect, such as Schmorl's nodes, is not rare in degenerated motion segments. 4) In the CE and SB region, there are ample sources for inflammatory cytokines from immune cells in blood, chondrocytes in CE, and cells in degenerating NP tissues. Therefore inflammation may exist in these regions and may be a possible source of low back pain. However, disc degeneration is generally found in older asymptomatic populations (60-80 yrs old) suggesting that simple degeneration is not sufficient to cause the back pain seen in DDD patients. There must be another extenuating factor that contributes to the pain seen in this disease. We believe that the introduction of the abnormal shear load would exacerbate the degeneration resulting in pain. According to the follower load theory, the back muscles play a major role in stabilizing the spine. It follows, then that the subchondral bone, cartilage endplate and the central portion of the disc would be subjected to these shear forces that may occur in the case of abnormal (temporal or chronic) control of back muscles.

2.6.6. Hypothesis and Aims

Discogenic low back pain is a mysterious entity. Previous studies have shown that disc degeneration can lead to instability due to greater segmental motion. However, the exact relationship between disc degeneration, instability, and low back pain remain unclear. Because of this ambiguity, our treatment strategies vary and do not always relieve pain. It becomes necessary, therefore, to 1) improve our understanding of the relationship of DDD and instability, and their relationship with pain 2) to develop better treatment strategies for this condition. In order to accomplish this goal, animal models of discogenic low back pain are needed. Currently, only one study has investigated this

issue. However, this study was conducted over a short period of time and included no definitive measures of pain in the discs as well as the spinal cord. Therefore, a reliable, reproducible animal model of discogenic back pain is needed. In this study, we hypothesized that the application of an abnormal shear loading to the lumbar spine will result in disc degeneration and discogenic low back pain. The aims of this study were as follows:

1. Creating disc degeneration within the spine resulting from the shear load.

   This was accomplished by attaching a novel spinous attachment unit to the L5 and L6 vertebrae. The shear load was applied to the L6 vertebrae.

2. Confirming disc degeneration

   Histology, in particular Hematoxylin and Eosin (H&E) staining, was used to microscopically analyze the intervertebral disc (IVD) for degenerative changes.

3. Confirming pain in rats

   Von Frey pain filament tests were used to monitor pain behavior in the rat. Immunohistochemistry was used to confirm pain by detecting the presence of the phosphorylation of creb (P-CREB) in the spinal cord and c-fos expression in the dorsal horn with focus on laminar areas 1, 2, 3, and 4.

4. Confirming discogenic pain

   Immunohistochemistry was used in the IVD and subchondral area to confirm discogenic pain. The nociceptive neurotransmitter Substance P (Sub P) was used to detect pain responses. Vascular Endothelial Growth

Factor (VEG-F), interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) were used to confirm the formation of new blood vessels (VEG-F) and inflammatory responses respectively.

CHAPTER 3

EFFECT OF SHEAR FORCE ON IVD DEGENERATION AND LOW BACK PAIN (MATERIALS AND METHODS)

3.1 Overall Research Design

In this study, we hypothesized that the application of an abnormal shear load would result in disc degeneration and low back pain. The goals of this study were to investigate whether shear loading resulted in sustained or increased disc degeneration when applied for longer than four weeks, to determine if pain would result from the disc degeneration, and to characterize the pain in terms of the signaling proteins in the spinal cord, inflammatory agents in the disc, and behavioral changes [Figure 8]. We postulated that the spine becomes unstable when there are deviations in the follower load path, and that shear force is a force that should be experienced in cases of instability. Therefore, we hypothesized that sustained shear force application would result in disc degeneration and pain that is comparable to that seen in DDD. To test this hypothesis, we used a novel shear loading device to create the abnormal shear force onto the rat spine for four and eight weeks. We then used histology to confirm disc degeneration and immunohistochemistry to confirm the presence of pain and discogenic pain. To our knowledge, only one study exists that investigates the effects of shear force application in relation to disc degeneration and low back pain. These preliminary results showed that disc degeneration was initiated within one week of shear force application and that pain behaviors were consistent with the onset of disc degeneration. The current study will

expand this knowledge using immunohistochemistry in conjunction with histology and pain behavior testing to discover the effects of shear load application for a more substantial time period.

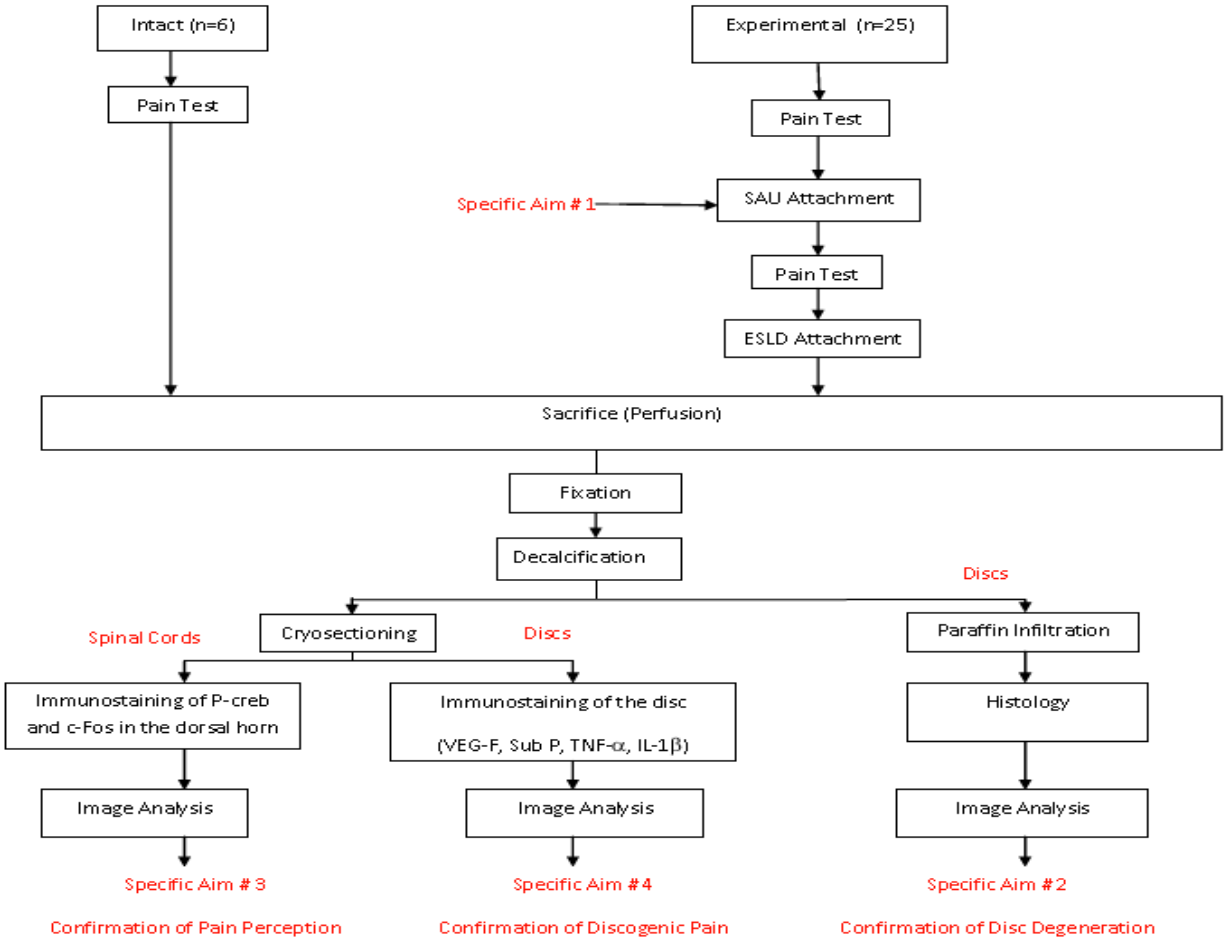

Figure 8: Details of Experimental Design

3.2. Animal Selection

Sprague Dawley rats were used in this study for the following reasons. First, the rat has similar anatomy humans and the histology of its IVD has been established and documented. Secondly, rats are small, relatively easy to handle, and cost efficient. Thirdly, rats have been used frequently in musculoskeletal research. Lastly, the rat IVD was shown to maintain normal histology at 6 and 22 months of age. Therefore, the IVD in the rats used are not starting the degenerative process [147].

3.3. Materials and Methods

A total of 55 Sprague Dawley rats (about 17 week old, 380-400g; Harlan, St. Louis, MO) were used in this study according to the protocol approved by the University of Iowa's Animal Care and Use Committee. However, a total of 24 animals had to be excluded from the study due to various unexpected emergencies (flooding in 2008 summer and the contamination in the University Animal Care Laboratory) and technical reasons (failure in using new shear loading device due to the breakage of spinous processes and the device failure during the follow-up period in some animals). As a result, a total of 31 animals were included in this study and assigned into one of the test groups listed in Table 3.

Group #	Animal#'s	Type of Group	Description of Treatments
1	6	Normal-Control	21-wk old and 25-wk old animals with no treatments
2	5	Immobilization-Control	Immobilization of L56 for 4 weeks
3	12	Experimental	Shear force application for 4wks
4	8	Experimental	Shear force application for 8wks

Table 3: Table of Experimental Design. Shown here are the numbers of animals in each group along with the description of treatments given.

3.3.1 Shear Loading Device

The stainless steel shear loading device was developed to apply a continuous static shear load of 4 N (similar to the animal's body weight) on the L6 vertebra of the rat spine in dorsoventral direction *in vivo*. The device consists of two spinous attachment units (SAUs) and an external shear loading device (ESLD) as shown in Figure 9. The SAU was designed in two different forms. The one for L5 vertebra had a saddle to firmly

hold the spinous process at the base with screw holes for the attachment of the ESLD on the other end. The other SAU for L6 had the same saddle as the L5 attachment, but had a dented end for adapting the tip of the piston in the external loading device. The ESLD consisted of a base frame and a cylinder in which a compressed spring pushed the piston against the dented end of the L6 SAU [Figure 10]. The cylinder was attached to the base frame using a revolute joint in order to allow the rotation of the cylinder in the sagittal plane. When ready, the loading piston was released and pushed the SAU on L6 downward. The initial spring force was fine-adjusted to achieve 4 N by changing the compressed distance of the spring using a set screw located on the top of the cylinder. It was found that the applied shear load, calculated based on the compressed distance of the spring measured immediately after euthanization at the pre-determined time point, varied within the range of – 0.8 and +0.4 N from the initial load set at the time of surgery.

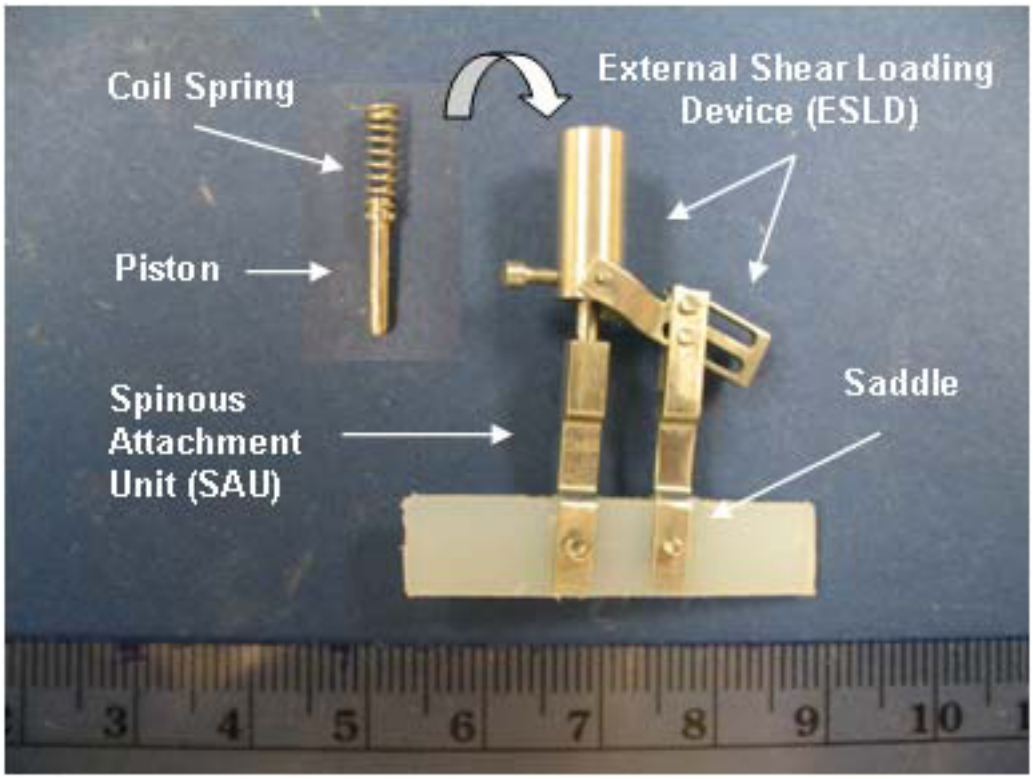

Figure 9: Shear loading device. The device consists of 2 spinous attachment units (SAUs) and an external loading device (ESLD). SAUs are designed to transmit the shear force generated by the spring inside the external shear loading device by being attached to the spinous process.

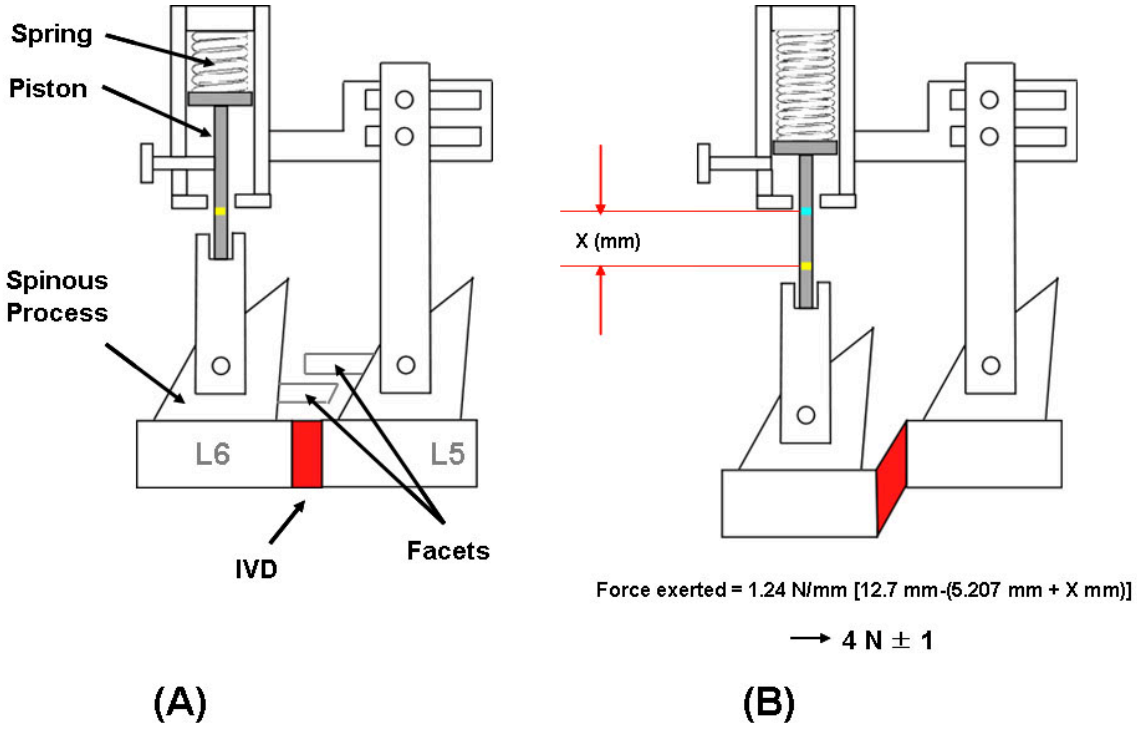

Figure 10: Diagram showing the function of the shear loading device. (A) External shear loading device was attached to the two SAUs with the piston in compression inside of the device fixed by the screw; (B) The screw was loosened and allowed the spring to uncompress, pushing down the piston, and resulting in shear load application on the disc. The uncompressed distance was measured by marking (shown in blue) and the shear force calculated by the equation given.

3.3.2. Procedures for SAU Implantation and ESLD Attachment

After inducing surgical anesthesia with isoflurane (5%) in air (400-450 ml/min), a midline incision (approx. 2 cm over the spinous processes of L4-S1) was made to expose the spinous processes of the L5 and L6 vertebrae [Figure 11]. Each SAU was fitted successively to L5 and L6 by gently compressing the saddle with a needle holder while keeping the vertical shaft aligned normal to the rat's body axis. A hole was drilled through the spinous process with a surgical drill while holding the SAU in place. The bolt threads into the other side of the saddle to fasten the SAU firmly to the spinous process, but not too tight to prevent necrosis. The wound was closed in layers. After surgery, Ketoprofen (10 mg/Kg, PO) impregnated Jell-O® was also provided once a day for three post-surgical days. After a 3 day recovery period, the rat with SAU attached was anesthetized, and the ESLD was mounted to the SAUs whose ends extruded from the skin. The ESLD was firmly attached to the top of the L5 SAU using bolts and nuts whereas the tip of the piston was placed into the indented hole in the tip of L6 SAU. Figures 11a and 11b show the experimental animals with SAU and ESLD attached.

Animals in the immobilization control group received the identical surgery except that two L5 SAUs were attached to both spinous processes of L5 and L6 vertebrae (Figure 12c). Three days after surgery, a solid plastic plate instead of ESLD was attached to connect the SAUs in order to immobilize the spinal motion between the L5 and L6 vertebrae.

Following the experimental follow up period, the animals were sacrificed by perfusion. Briefly, the rats were first anesthetized with sodium pentobarbital. Next, the heart was exposed and the aorta flushed with hepranized saline. 1000mL of

paraformaldehyde was then infused for 30 minutes to ensure proper fixation. The spines were dissected and prepared for histology and immunohistochemistry.

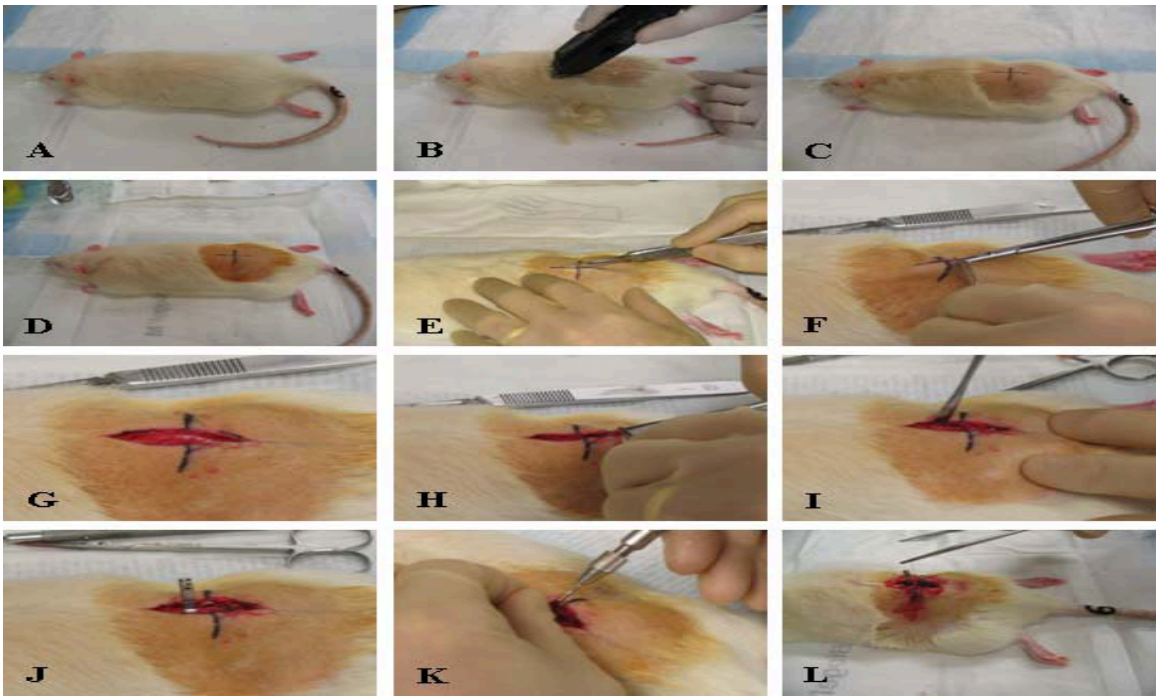

Figure 11: Surgical procedures for SAU implantation in rats. (A) a rat anesthetized with isoflurane; (B) hair removal from the surgical area; (C) identification of L6 spinous process by palpating the iliac crests and a guide line drawn over the spinous process of L6; (D) disinfection of the surgical area with iodine; (E.F) skin incision made over a guideline, approximately 2 cm; (G, H) incision of subcutaneous fascia; (I) incision of lumbo-dorsal fascia and separation of paraspinal muscle from both sides of the L5 and L6 spinous processes using Freer chisel; (J) placement of front SAU on L5 spinous process to be used as a drill guide; (K) drilling to secure the SAU to the spinous process; (L) completed implantation of the front SAU on L5 spinous process after the screw tightened. Procedure is identical for the rear SAU implantation on L6 spinous process

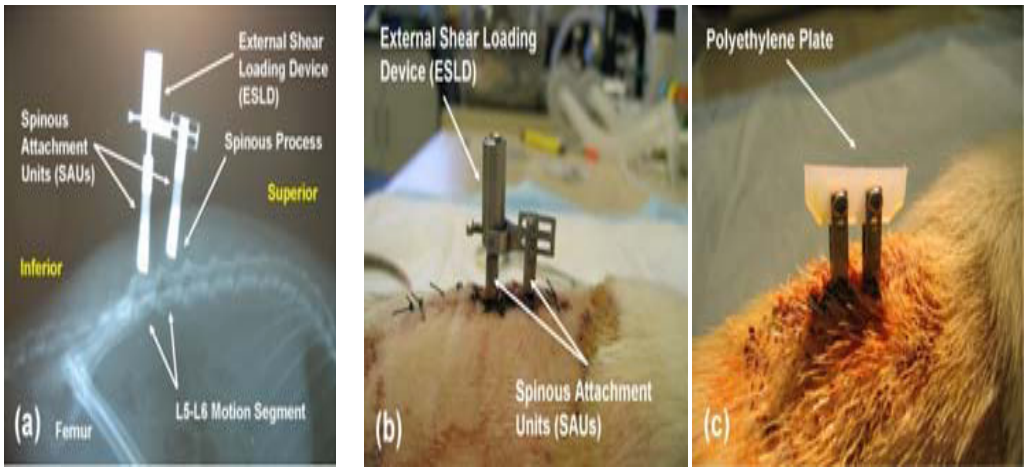

Figure 12: Image of a rat with shear loading device surgically attached to the spinous processes of L5 and L6 vertebrae through SAUs. (a) X-ray image of the device. The longer SAU was attached to the spinous process of L5 and the shorter one to L6. (b) Part of SAUs and external shear loading device are shown. (c) Polyethylene plate attached to the two L5 SAUs for immobilization control group.

3.3.3. Pain Behavior Testing

3.3.3.1. Animal Preparation

Housing of animals: Animals were kept in a 12-hour dark-light cycle at a room temperature of 22-24°C, with free access to food and water, and were allowed at least 10 full days from the date of delivery by the supplier before animals are used for behavioral experiments.

Acclimatization: Acclimatization of the animals to the testing environment is important. Before testing, animals were allowed to acclimate for at least 15min for rats, in the cubicle placed on the platform, where the actual testing is done. Careful attention was made to ensure that the animals were in a calm environment in which noise was kept at a minimum. No other animals or people were allowed to distract the tested animal. These precautions were taken to be sure that the animal was not stressed in any way.

3.3.3.2 Mechanical Withdrawal Testing

Pain behavior was measured in terms of mechanical withdrawal threshold (MWT) of both hind paws using Von Frey Filaments (Touch Test®). The tests were performed at following times: before surgery (baseline), 1 wk, 2 wk, 4 wk, 6 wk, and 8 wk after implementation of a plate for immobilization control group and an ESLD for experimental group. Rats were placed in clear plastic cubicles on an elevated wire mesh plate (15" X 19" X 10.5") to ensure accessibility for the tester and allowed to acclimate for 15 min [Figure 13]. von Frey monofilaments (North Coast, CA) of varying bending forces (10 - 495 mN) were applied to the plantar surface (as shown in Figure 14) in ascending order starting with the lowest. Two trials per filament were performed, and the paw had to lift for two sequential filaments for the force to be recorded.

Testing took place during the day when the animals were alert in a blinded manner. Each filament was calibrated prior to testing. Intact normal rats generally do not respond to force below 187mN, and a response below that force is considered a painful response.

Pain behavior data was analyzed using non parametrical methods. For differences between the treatments within the group, the Friedman repeated measures on ranks test were conducted. For differences between the groups, the Kruskal-Wallis one way ANOVA on ranks test was used. The Mann-Whitney test was used in cases where there were two groups to compare.

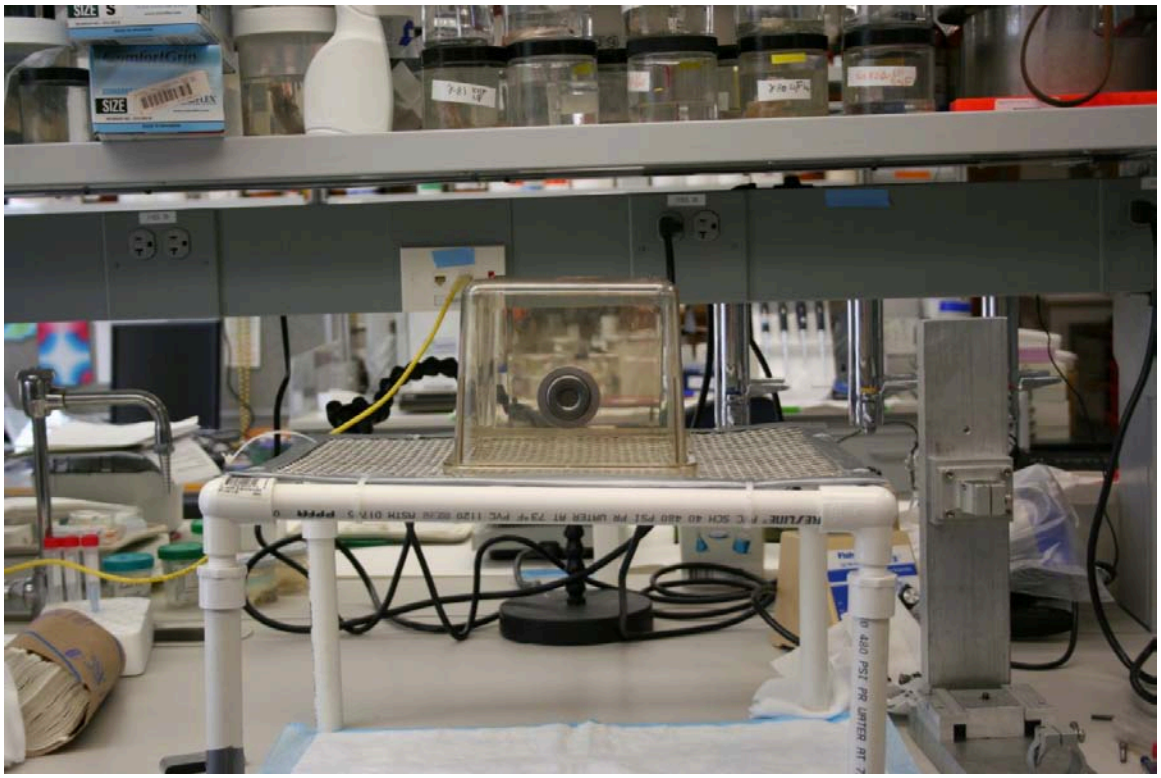

Figure 13: Mechanical Withdrawal Testing Set Up. The wire mesh plate is shown along with the caging to contain the rat.

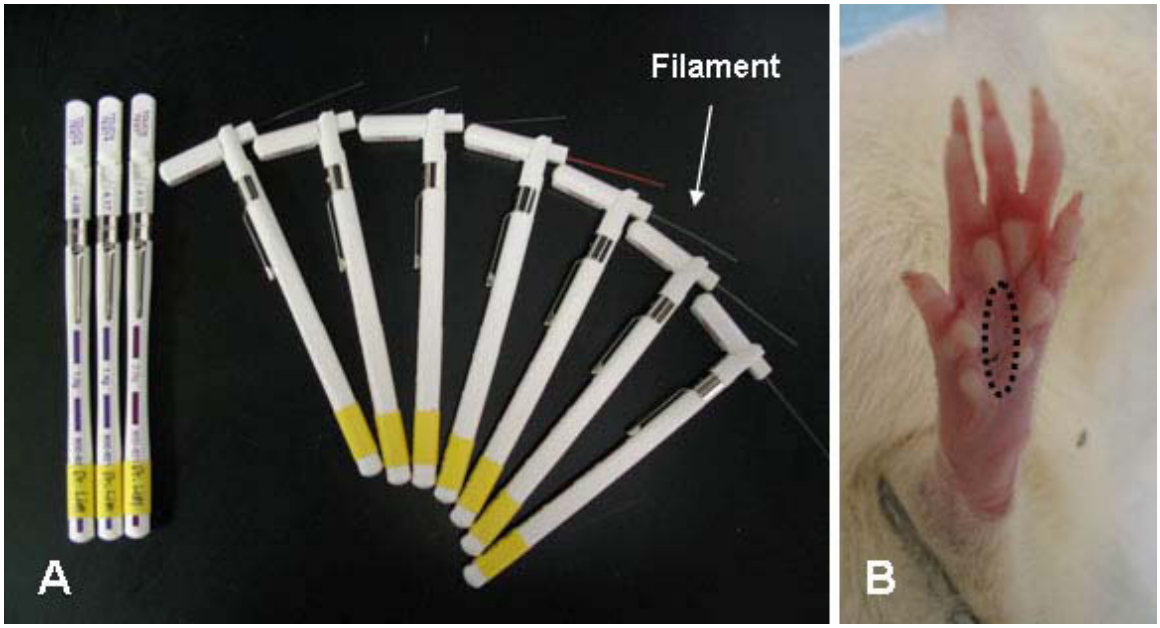

Figure 14: Filaments and Area of Application. (A) Von Frey filaments of varying bending forces. (B) The plantar surface of the rat hindpaw.

3.3.4. Immunohistochemistry Testing

Immunohistologic analysis was used to detect pro-inflammatory cytokines (TNF-α and IL-1β), nociceptors (substance-P), and neovascularization (VEGF), which are linked to discogenic pain and disc degeneration [148, 149]. Frozen sections through the midline of intervertebral disc were stained by indirect immunofluorescence according to standard protocols. Monoclonal antibodies specific for rat substance-P, TNF- α, IL-1β, and VEGF were diluted as described by the antibody suppliers. Goat anti-mouse-Alexafluor 568-conjugated secondary antibodies were used for detection. Negative controls were included, in which only the secondary antibody was applied. Rat dorsal root ganglia were used as positive controls for substance P. Slides were mounted in Vectashield containing DAPI, a UV-excitable nuclear stain. Epifluorescence imaging was performed on an Olympus BX60 (Leeds Precision Instruments, Inc., Minneapolis, MN) microscope equipped with a digital camera (QImaging Qicam). Each field was imaged using 568 nm excitation (immunopositive cells) followed by 410 nM (UV) excitation (nuclei of all cells).

Immunohistochemical analysis of the dorsal horn of the spinal cord was performed to detect the presence of c-Fos and P-creb. c-Fos is a known to signal pain and P-creb is phosphorylated by c-Fos. The rat spinal cord was harvested, cryoprotected, and frozen in a -80º freezer. The cord was then cryosectioned into 10μm sections and mounted on a slide for immunohistochemistry. Antibodies specific for rat c-Fos and P-creb were diluted as specified by the suppliers as shown in Figure 15. Goat anti-mouse-Alexafluor 568-conjugated secondary antibodies were used for detection. The sciatic

nerve of a sciatic rat was used as a positive control. Exactly as before, slides were mounted, stained, and imaged.

Figure 15: Immunohistochemical analysis process. Slides are shown with antibodies placed over spinal cord sections.

3.3.5. Histology

Immediately after the perfusion and removal of the spinal cord, the lumbar spine (L4-S1) was carefully harvested from each animal. The harvest samples were cleared of all muscle tissue and ligaments using surgical scissors and scrapers. They were then placed in 5% formic acid solutions for decalcification. The samples were left in solution for a minimum of two days and were checked to ensure total decalcification. After decalcification, the samples were cut into motion segments consisting of two vertebral bodies and the intervertebral disc using a straight blade. Cuts were made transversely starting with the L4 level and ending with S1. Once the motion segments had been isolated, each segment was then cut into half through midsagittal plane. One side (alternatively picked) was embedded into paraffin for histology while the other side specimen was preserved at -80°C for the immunostaining of the disc as described in immunohistochemistry section.

The prepared paraffin block of the half of the motion segment was cut into 5 μm sections and stained with hematoxylin and eosin (H&E). The H&E sections and graded by two independent investigators for signs of degeneration in the AF, NP, cartilage endplate and subchondral bone using the scale suggested by Gries et al[150]. Briefly, the changes in each region were graded from 0 (normal) to 3 (severe) as shown in table 4. The histology pictures in Figure 30 were selected from the results of current study. The mean values of these gradings obtained from two observers were used for analysis. We used Kruskal-Wallis one way analysis of variance on ranks and the post hoc Dunn's method for statistical analysis.

Grading Scale	Tissue	Histological Appearance
0	NP	Elliptical cavity with well defined borders Thin matrix, notochordal cells in organized pattern
	AF	Thick, straight lamellae anteriorly, curvature posteriorly
1	NP	Notochordal cells less aggregated, some chondrocytic cluster formation, less transparent matrix
	AF	Concentric structure still visible with crack formation
2	NP	Fibrocartilaginous matrix, less distinction between other structures, notochordal cells depleted, increase in chondrocytic cluster
	AF	Intrusion of AF into NP space, less organized laminar structure
3	NP	Fibrocartilaginous matrix, no distinction from surrounding structures, chondrocyte-like cells dominate space, flat appearance
	AF	Little discernment between NP and AF, cracks, tears, fissures permeate the structure

Table 4: Grading Scale. Adopted from Gries, et al., this grading scale distinguishes the changes in the IVD in terms of the nucleus pulposus and annulus fibrosus.

CHAPTER 4

EFFECT OF SHEAR FORCE ON IVD DEGENERATION AND LOW BACK PAIN (RESULTS)

4.1. Results

4.1.1. Surgery of SAU implantation

In all, 24 rats were excluded from the study due to contamination in the lab, the flood in Iowa City in June of 2008, and failure of the device during the follow up period. The surviving rats all survived the surgery without complication. The rats were monitored closely for signs of loss of appetite, loss of weight, and physical signs of distress (i.e. cessation of grooming habits). All rats gained weight consistently and remained physically healthy throughout the follow up period.

4.1.2. Effect of Shear Load on Low Back Pain

4.1.2.1 Pain Behavior Analysis

To eliminate the variations in the intact animals before surgery, the mechanical withdrawal testing (MWT) values measured before surgery were used to determine the baseline mean value. The changes in mean MWT values of each group at different follow-up time points from the baseline mean MWT value are shown in Figures 17 and 18.

Pain responses were found in the experimental groups (3 and 4) but not in the intact control group 1 and the immobilization control group 2 as shown in Figures 16 and 17. In the control groups 1 and 2, the average bending force changed overtime somewhat, but the MWT changes over the 8 week period were not significantly different ($p > 0.9$). In contrast, MWT values in shear loading groups 3 (4 week) and 4 (8 week) showed significant decrease from the baseline MWT value 1 and 2 weeks after loading and remained below the pain criterion of 187 mN. These changes clearly showed mechanical hyperalgesia resulting from shear loading. Figures 16 and17 show the pain responses that were measured over the course of the experiment for the control groups (control and immobilization control) and the experimental groups (4 and 8 weeks shear loading) respectively.

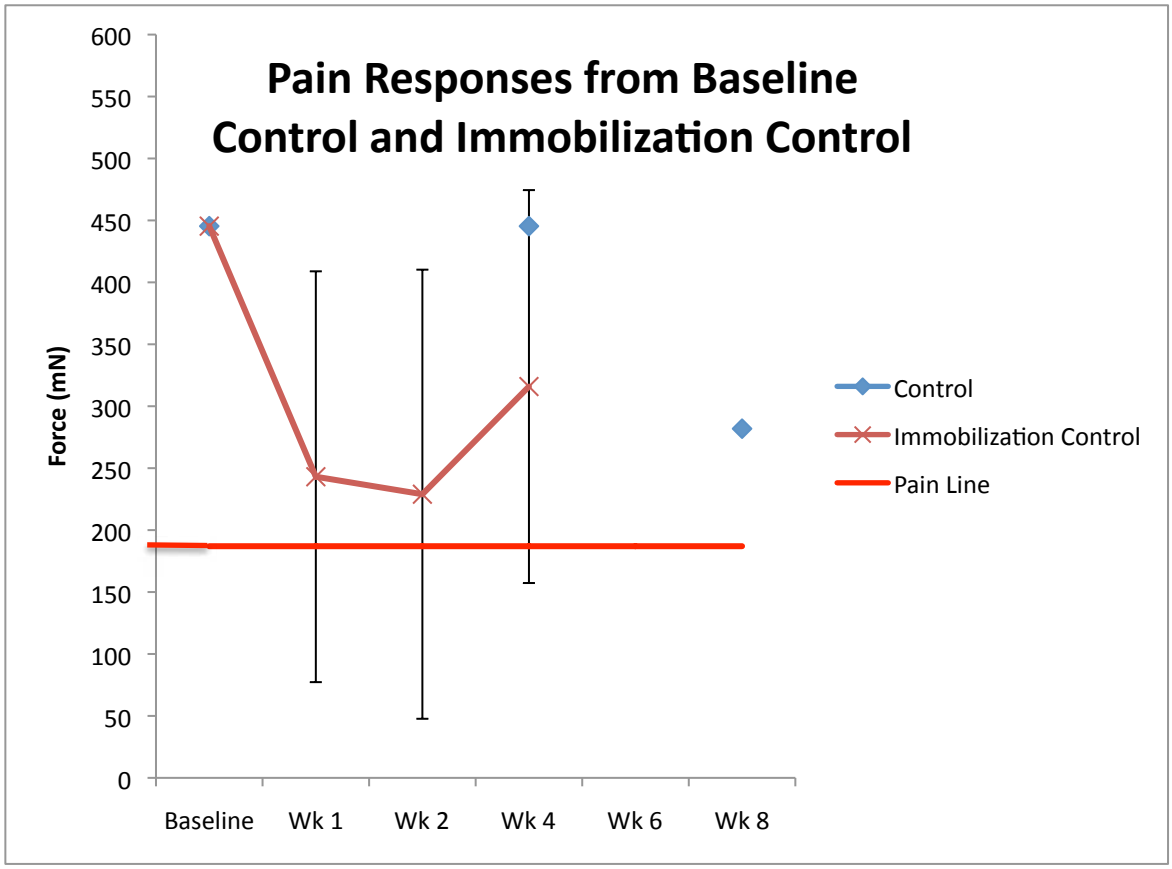

Figure 16: Pain behavior responses according to group. The pain line indicates that responses below this value are considered painful.

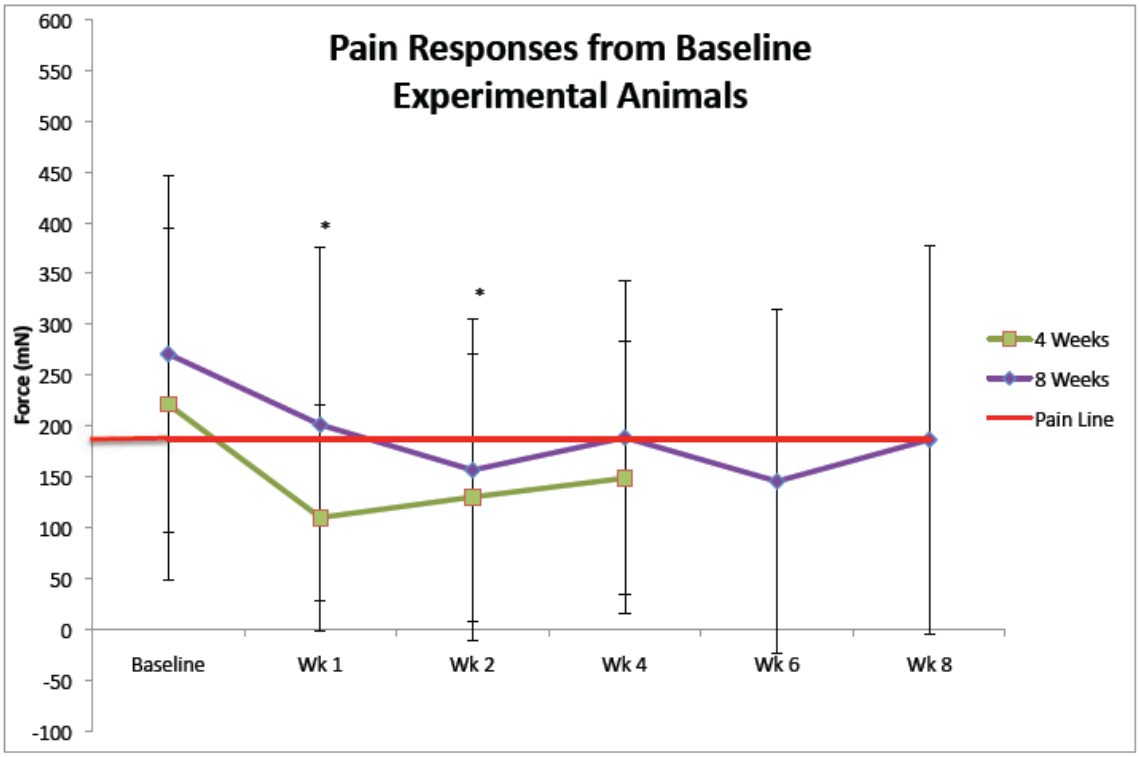

Figure 17: Experimental group pain response behavior. The pain line indicates that responses below this value are considered painful.

4.1.2.2 Immunohistochemical Analysis: Pain in the Disc

Although the specificity of antibodies to rat TNF-α , and IL-1 as immunohistologic probes is well-documented, little or no positive staining was observed

in NP, AF, or EP of discs from any group. Heavy positive staining of dorsal root ganglia antibody confirmed the specificity of the anti rat substance-P antibody. However, the antibody failed to detect substance-P in intervertebral discs. In contrast, VEGF was present in and around blood vessels in vertebral bodies from control and experimental samples. The percentage of VEGF-expressing cells (anti-VEGF positive/total nuclei x 100) differed according to level and was slightly lower in experimental samples than in controls. Taken together these results indicated no significant effect of loading on pain- or inflammation-related markers. Figures 18-24 show the positive control samples along with the corresponding negative staining experimental animals.

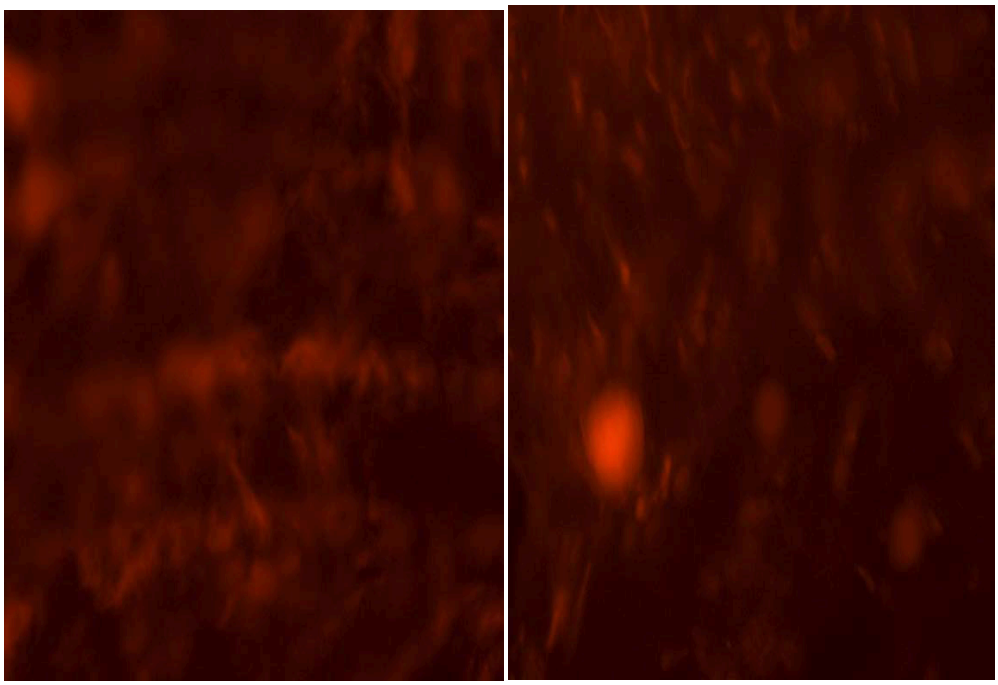

Figure 18: VEG-F positive control (pictured on the left) and positive staining in the vertebral bodies (pictured on the right) shown at 10X magnification.

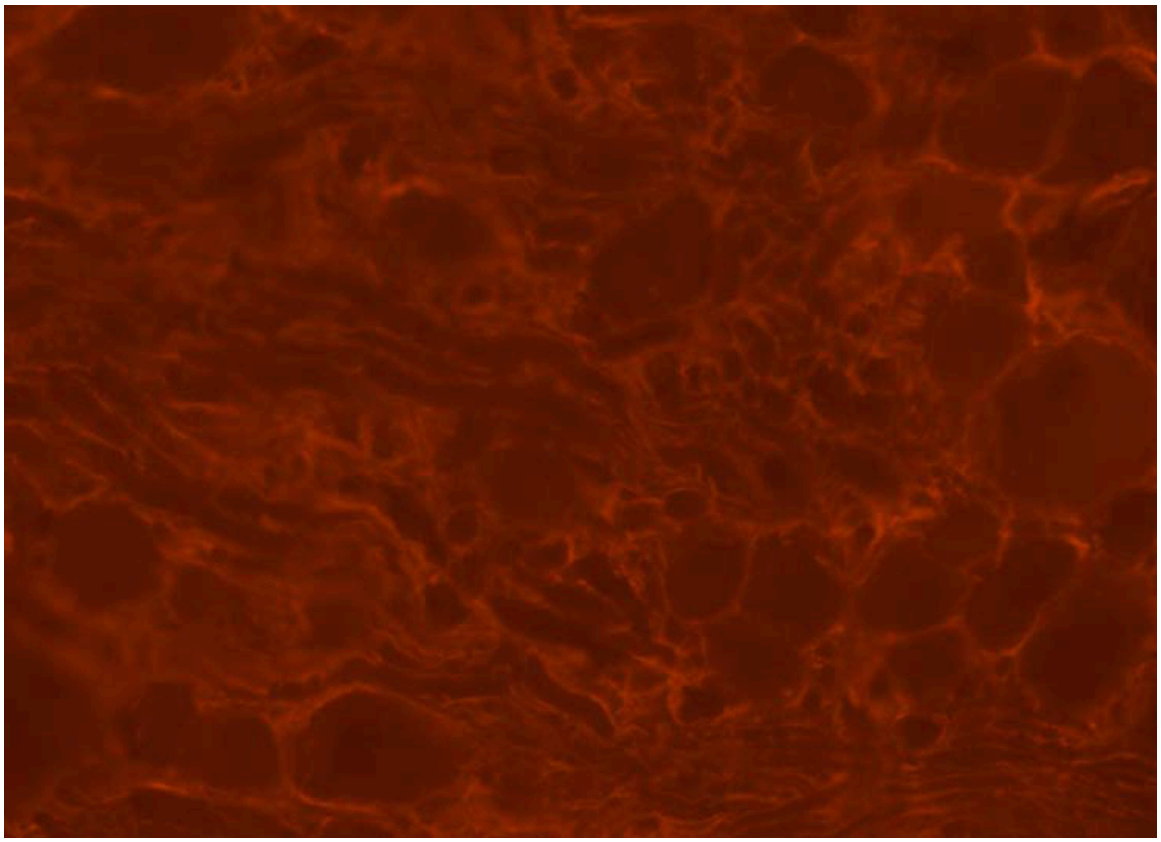

Figure 19: Positive control staining for Substance P (10X magnification).

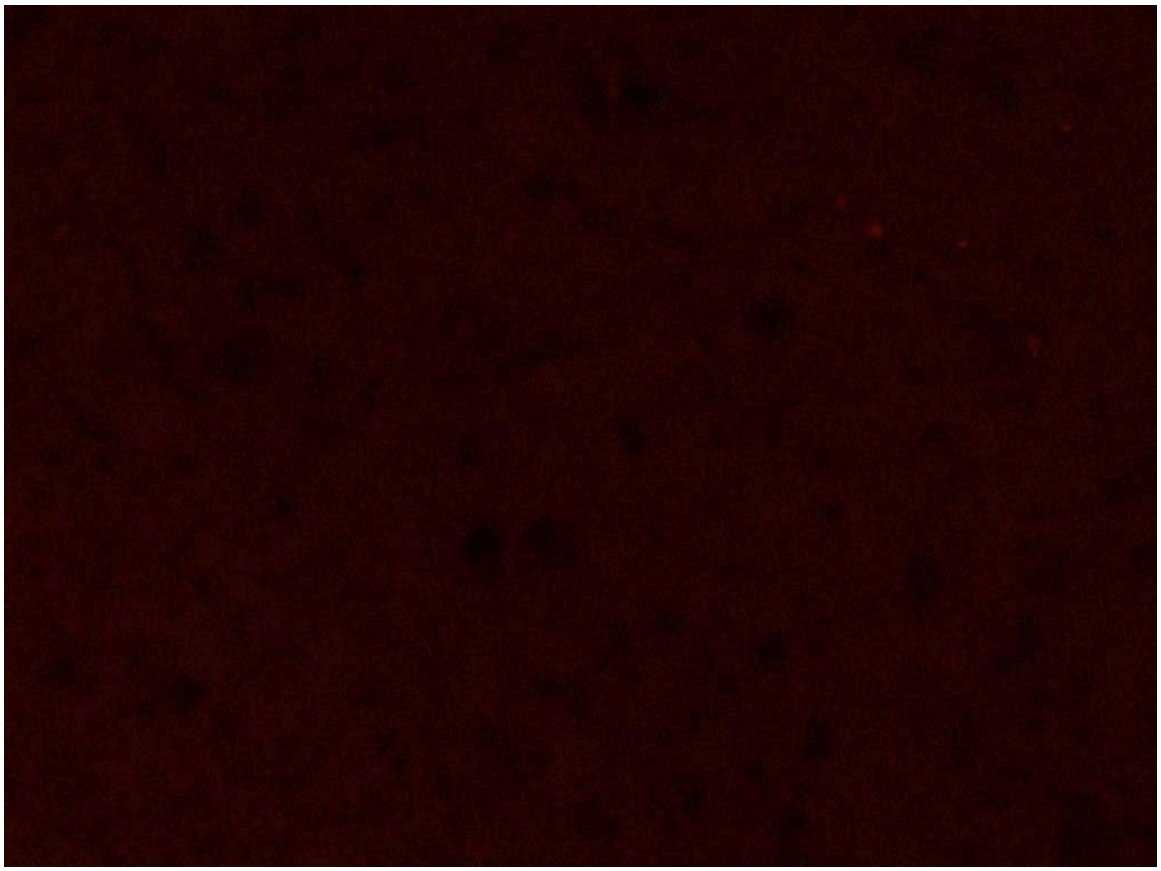

Figure 20: Negative staining for Substance P in the IVD (10X magnification).

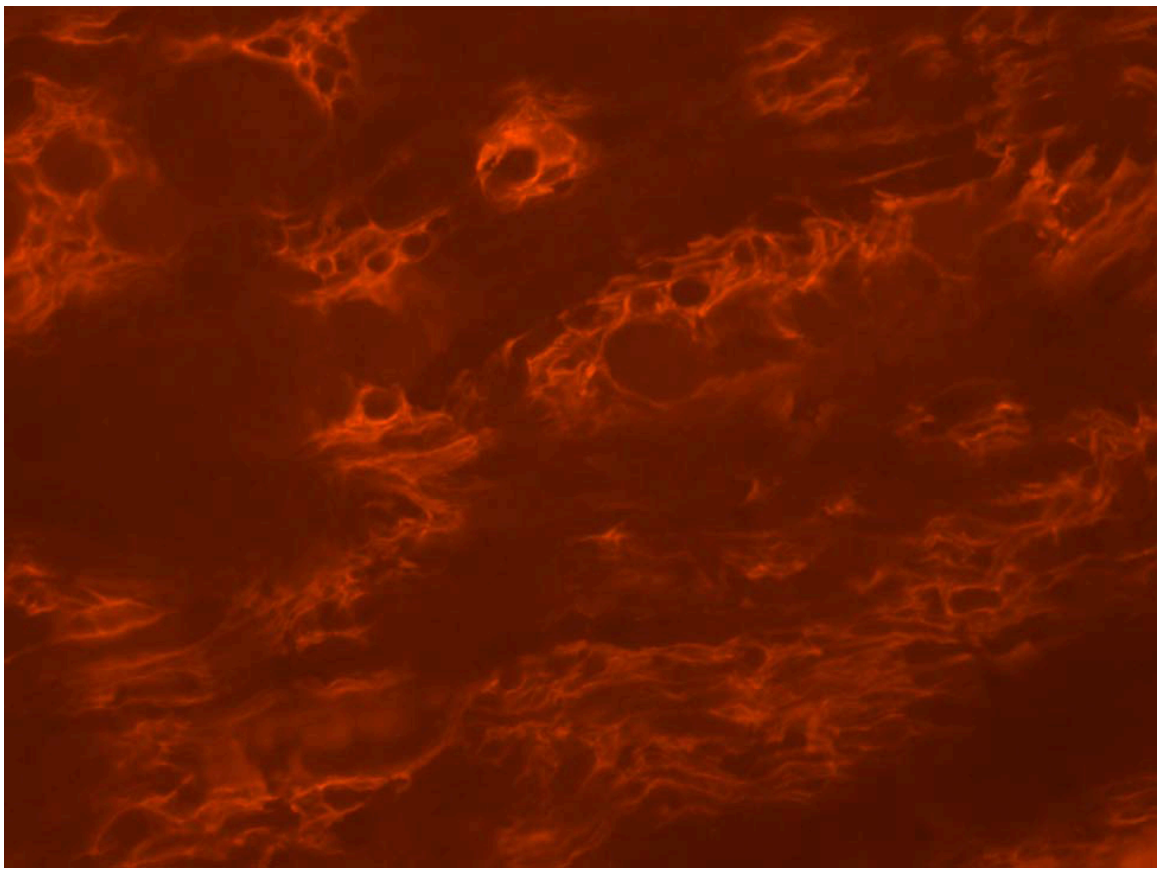

Figure 21: Positive control for TNF-alpha (10X magnification).

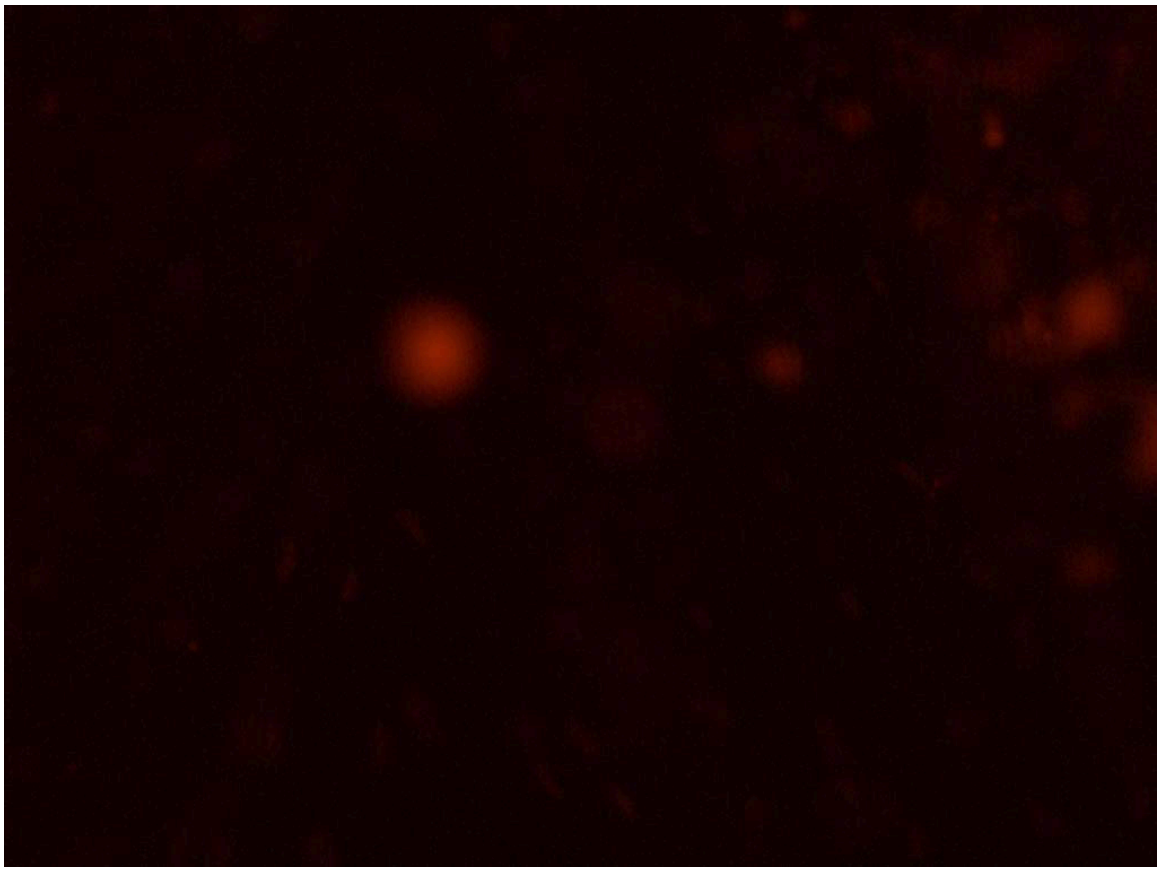

Figure 22: Negative staining in the IVD for TNF-alpha (10X magnification).

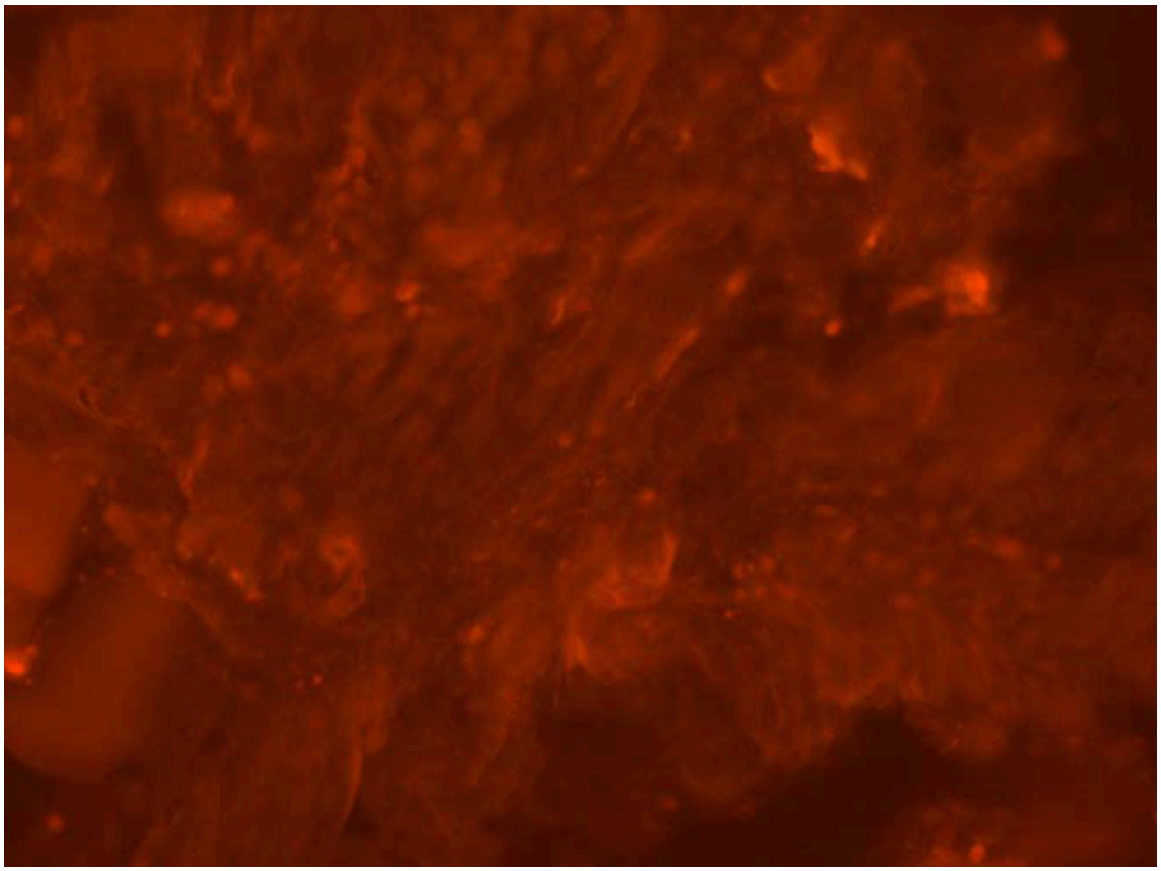

Figure 23: Positive control staining for Il-1beta (10X magnification)

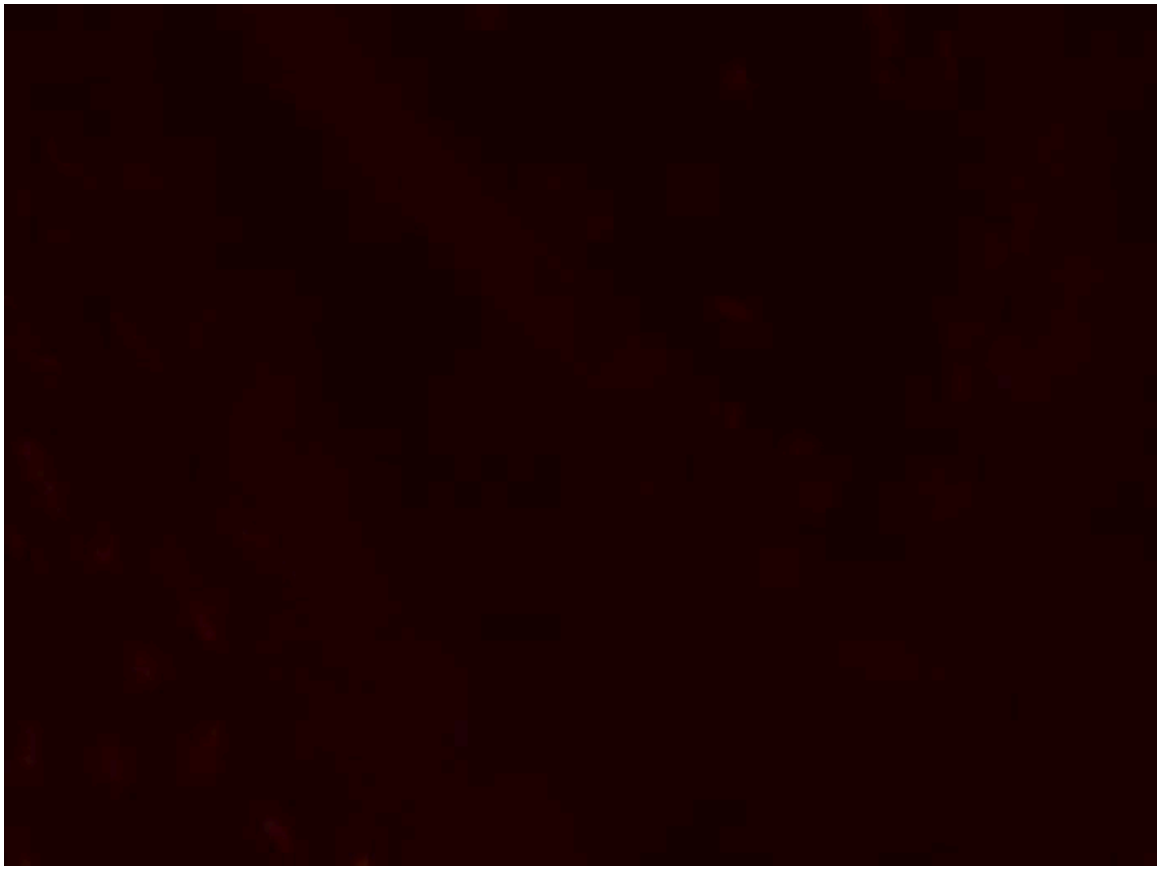

Figure 24: Negative staining in the IVD for Il-1beta (10X magnification).

4.1.2.3. Immunohistochemical Analysis: Pain evidence in the Spinal Cord

Little to no positive staining was found in the dorsal horn for the c-Fos and P-creb antibodies. Heavy positive staining of sciatic nerve positive control confirmed the specificity of the c-Fos and P-creb antibodies. The majority of positive staining that was seen at lower magnifications (40X) were identified as auto fluorescence at higher magnifications (800X). Of those that were indeed positive, the ratio of positive/negative staining was negligible. Figures 25-28 show the positive control samples for each antibody used along with the negative staining obtained from the experimental and control samples.

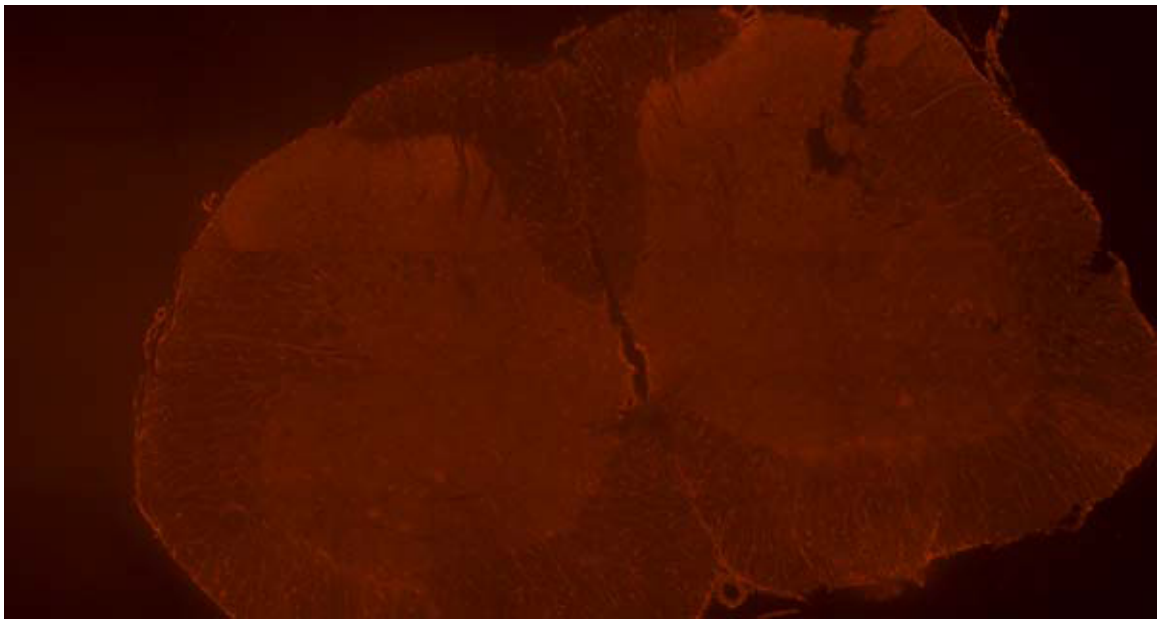

Figure 25: Positive control staining for c-Fos antibody (10X magnification).

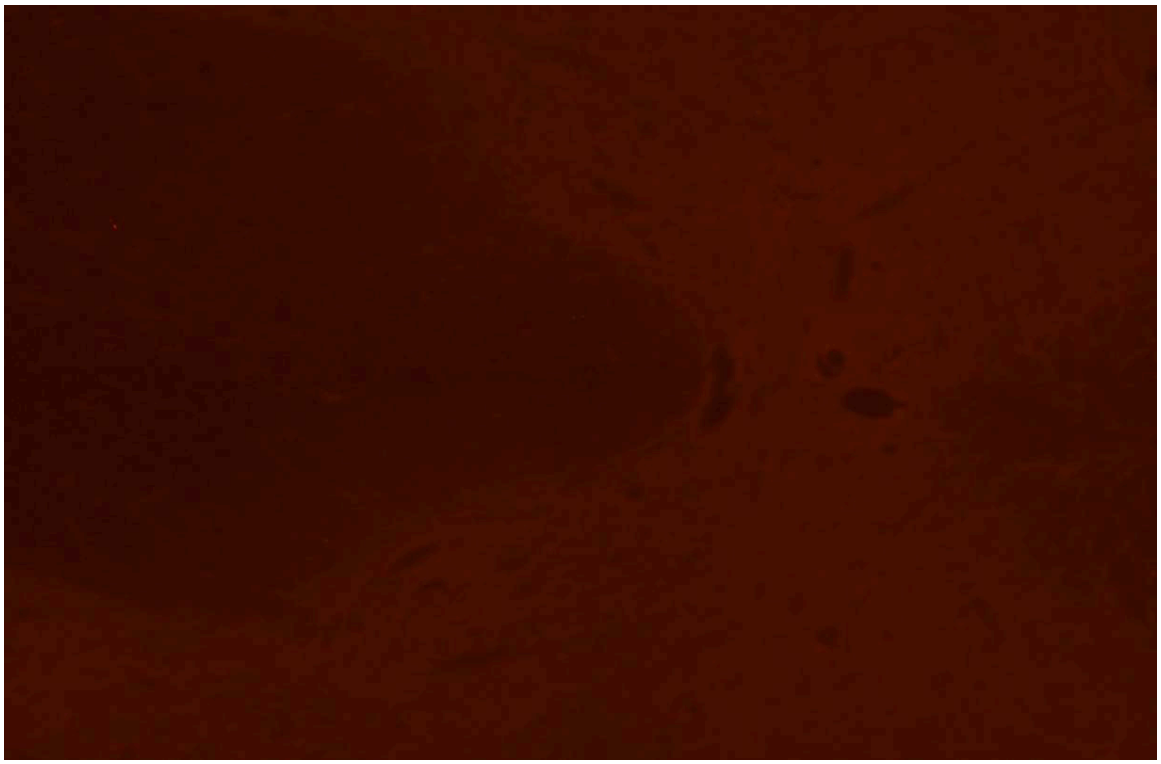

Figure 26: Negative staining in the dorsal horn for c-Fos (40X magnification).

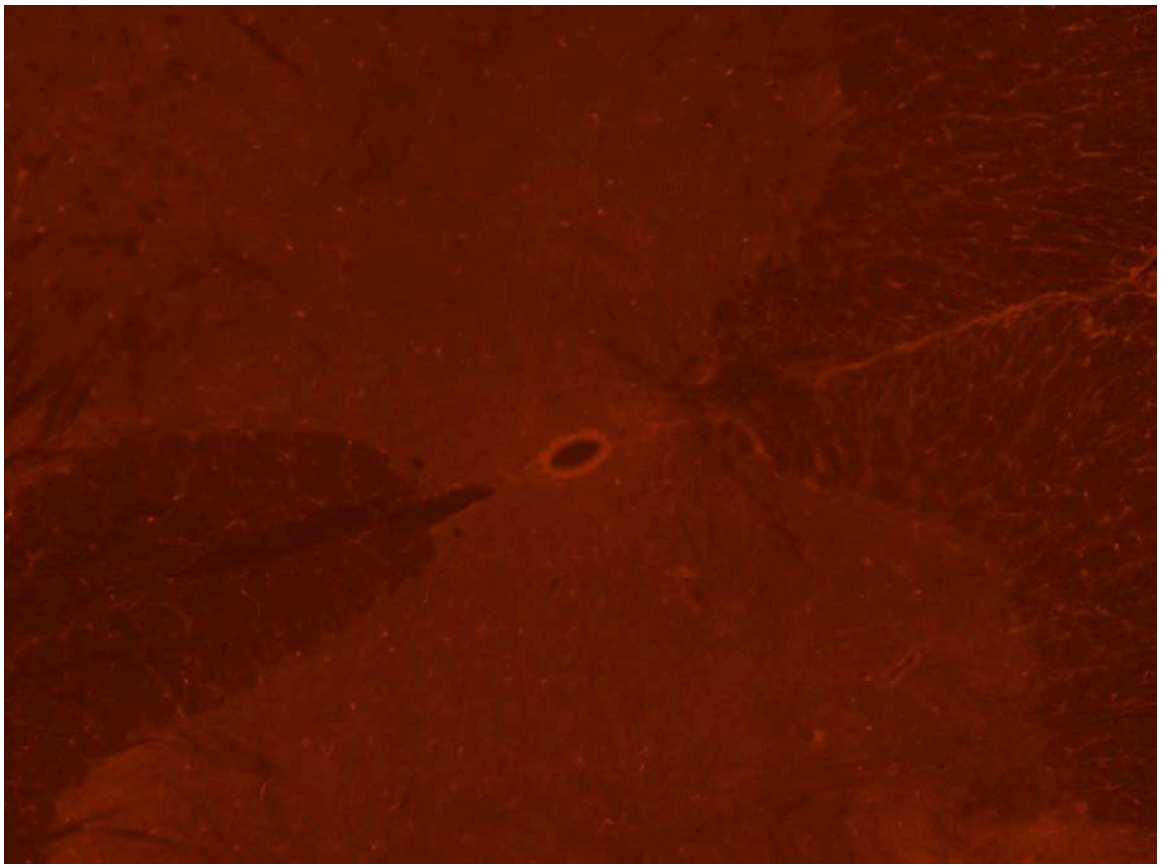

Figure 27: Positive staining for the P-creb antibody (40X magnification).

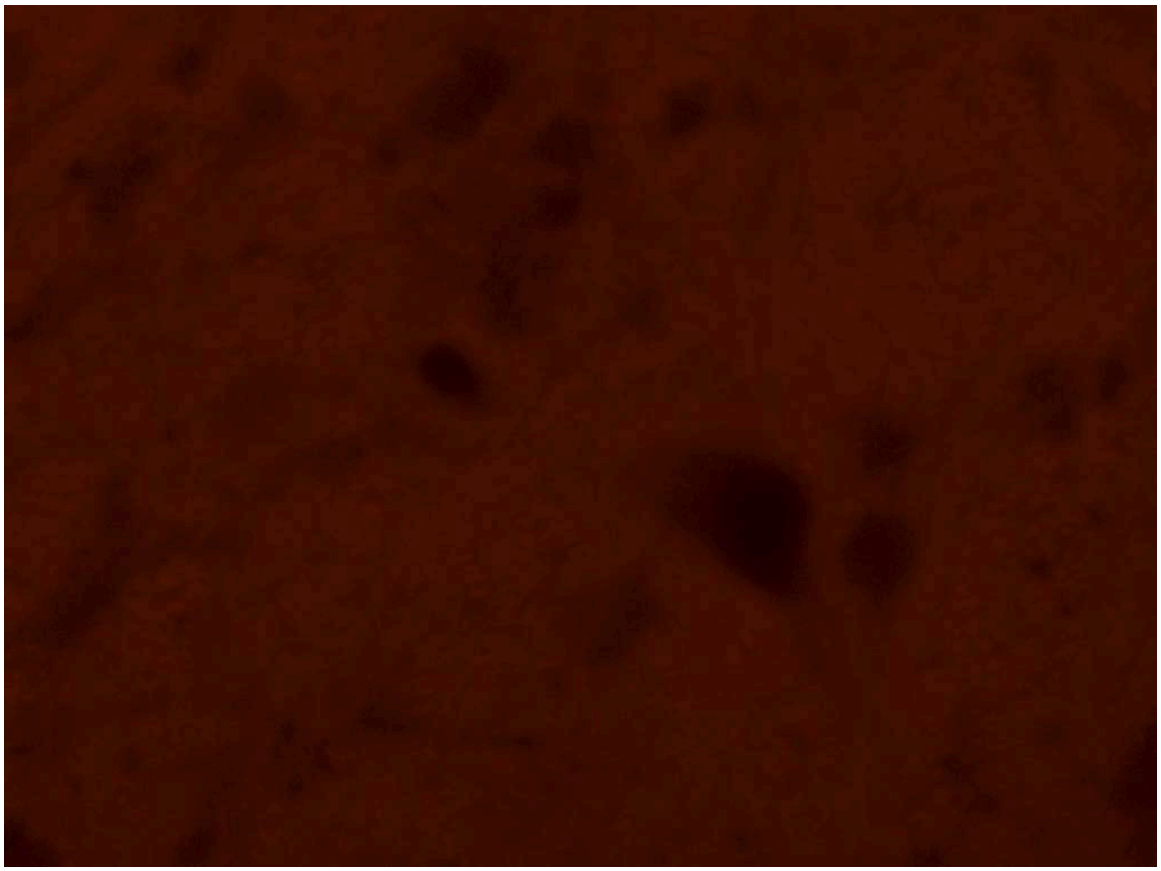

Figure 28: Negative staining for the P-creb antibody in the dorsal horn (40X magnification).

4.1.3. Effect of Shear Load on Disc Degeneration: Histological Analysis

4.1.3.1. Histology of the Normal Rat Disc

The normal rat disc contains a nucleus pulposus that is encapsulated and distinct from the annulus fibrosus and the cartilaginous endplate. It is nestled in the posterior half of the disc and contains rounded notochordal cells. The notochordal cells are arranged in a long elliptical cluster formation surrounded by a thin transparent matrix. The annulus fibrosus encircles the nucleus pulposus with concentric rings of lamellae separated by fibrocartilaginous matrix between the layers. The anterior portion of the AF displays a relatively straight thick lamellae arrangement whereas posterior portion had thin lamellae that curved dorsally. The AF is attached to the firmly to the endplate on the anterior and posterior sides. The cartilaginous endplate is thick and filled with chondrocytic cells arranged in a regular pattern. Vertical chondrocytic cell lines can be seen extending from its border directing downward toward the NP/AF interface. The normal cartilage endplate is devoid of osteophytes.

Only two samples in our study were normal (grade 0). All discs in the immobilization control group showed grade 1 degeneration as did those in the intact control group while one showed grade 3 degeneration. All experimental animals displayed degeneration. Grade 3 degeneration was seen in all levels of the four week loading group and in two levels of the eight week loading group. More than 50% of the discs in both experimental discs showed degeneration scores of grade 2 or higher. No significant differences were found between the three motion segments for any group as well as between the intact and immobilized control groups. However, significant

differences were found between the control and four week group as well as the eight week group. Featured in Figure 29 are the actual samples obtained from our study according to the grading scale used in this study.

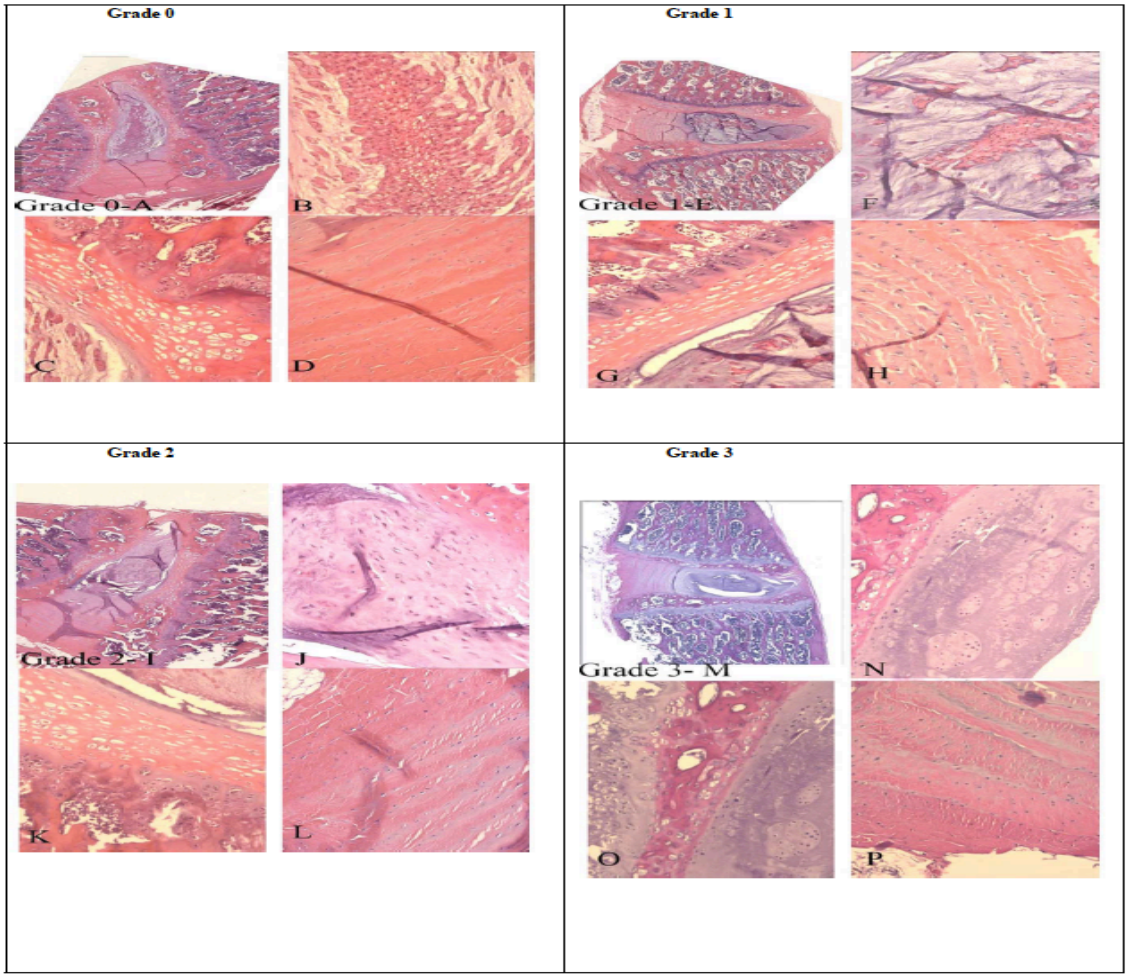

Figure 29: Samples of disc degeneration in this study. Grading scheme; A- motion segment; B -notochordal cells, thin matrix; C- Endplate, cells consistent; D- AF, organized, attached to the EP; E-motion segment; F- NP notochordal cells dying, replaced by clusters, matrix more dense; G-EP consistent; H- AF normal appearance; I-motion segment; J- NP more chondrocytic clusters, matrix denser; K- EP scattered small cells and larger cells present; L-AF cracks ; M- Entire motion segment with flattened appearance; N- Fibrocartilaginous NP, no distinction between NP and AF; O-Cartilaginous EP; P- AF cracks and tears

CHAPTER 5

DISCUSSION

An *in vivo* rat study was conducted to investigate the effect of sustained shear force application on the lumbar spine, on disc degeneration, and pain behavior. Histologic observation of the IVDs in all shear loaded animals for 4- and 8-week follow-up periods clearly showed that degenerative changes occurred at the loaded and adjacent disc levels as observed in our previous study[128] for the duration of 1 and 2 weeks. Prolonged duration of shear load application, however, did not produce more severe degenerative changes. Quantitative analysis also showed that the mean degeneration score of 1 and 2-week shear loaded groups at each disc level did not show significant differences compared with ones of 4- and 8-week shear loaded groups (data not shown). For the immobilization control group, prolonged immobilization for 4 weeks produced moderate degenerative changes (Grade 2) in 3 discs in our previous preliminary study, which was not found in the spines immobilized for shorter duration (1 and 2 weeks). All the discs in the immobilized control group of this study showed grade 1 degeneration.

The immobilized group served in this study as a control to confirm that disc degeneration resulted from the application of the shear load but neither from the immobilization nor from the surgical introduction of spinal attachment units (SAUs). The shear loading device was designed to allow flexion-extension motion of the L5-L6 motion segment but prevents the segmental motions in other directions. Such motion restraints may induce early degeneration of the IVDs at the immobilized level as well as

at the adjacent levels [151-153] while another study showed just few signs of degeneration under the similar immobilized condition [154]. Although identical segmental immobilization was not provided as the shear loading device in the absence of the spring, the results of our previous and current studies excludes the possible roles of immobilization or the introduction of SAUs on disc degeneration found in the experimental animals. Furthermore, the recent report of Zhang et al. [155] showed no significant morphological degenerative changes of the NP with no fibrosis in the aged (6 and 22 month) Sprague-Dawley rats. As such, the results of this study clearly demonstrate that the IVD degeneration (fibrosis of NP) found in the experimental groups occurred primarily due to the application of shear load.

IVD degeneration is multifactorial disorder, and excessive or abnormal mechanical stress is considered to be one of the major contributing factors in regulating cell metabolism in healthy as well as in degenerated IVDs. This hypothesis is supported by numerous *in vivo* studies [156-166] designed to investigate the effects of mechanical loading on the IVD. Certain static and dynamic compressive loads initiated degeneration and altered the composition of the disc in a load dependent manner. The results of these studies have suggested that a physiological window exists within which the IVD maintains its physiological functionality *in vivo* [167-169]. Particularly, Lotz et al. [170, 171] showed that the IVDs in the mouse tail degenerate when subject to compressive stress greater than 0.8 MPa but not in response to compressive stress less than 0.8 MPa. In our study, the rat lumbar discs degenerated in response to the shear force of 4 N (corresponding to the shear stress of approximately 0.33 MPa with the average cross-sectional area of the L5-L6 IVD of the rat lumbar spine as 12 mm^2) in one week. It is

interesting to note that the degenerative changes that resulted from the application of a shear stress (0.33 MPa) is much less than a critical compressive stress (> 0.8 MPa) found in the previous studies. Even though the direct comparison between the effect of compressive and shear stresses in these studies may not be appropriate, the degenerative changes in response to a lower stress observed in this study may indicate the greater detrimental role of shear stress than the compressive stress.

The L5-L6 segment was chosen as a shear loading site in an attempt to produce disc degeneration in the segment most equivalent to the L4-L5 in humans at which disc degeneration associated with LBP used to be observed most frequently. The results, however, showed that the degenerative changes occurred not only in the L5-L6 disc but also in all adjacent levels (L3-L4, L4-L5, L6-S1) although more advanced changes were found in L5-L6 and L6-S1 discs than in L3-L4 and L4-5 discs. It is fundamental in mechanics that, when subjected to a local shear force, a beam with both ends fixed experiences internal shear force throughout its whole length. As our shear loading device applies the dorso-ventral force to L6 vertebra, it is evident that all the adjacent levels should be subjected to the shear force of same magnitude in theory if there are no other external forces applied on the lumbar spine. *In vivo*, however, such external forces exist in the complex form of unknown muscle forces, thus it is impossible to determine the exact magnitudes of shear forces exerted on the adjacent levels, but it is reasonable to expect that all lumbar discs are subjected to a shear force of certain magnitudes. From this mechanical reasoning, the degenerative changes observed in the adjacent levels confirm the successful application of the shear load by ESLD and demonstrate the effect of shear force on disc degeneration. Furthermore, as shown in the Appendix, it was

found possible for the prolonged shear loading on the L5-L6 segment for 8 weeks to result in the scoliosis curve development in the thoracolumbar spine region. It is our postulation that abnormal spinal muscle contractions induced for adapting the abnormal shear force in the lumbar spine (L5-L6 segment) are likely to produce abnormal loads on the whole spine and to produce the scoliosis observed in this study during the growth of vertebral bodies. However, further studies to identify the muscle groups contracting abnormally due to the shear force application are required for proving this postulation.

A number of animal models for the study of disc degeneration have been introduced, and various methods have been used to induce disc degeneration in many studies. For example, either the injection of chemonucleolytic agents (such as chymopapain and chondroitinase ABC) or the physical damage in the annulus fibrosus (puncture or stabbing) was found to induce disc degeneration in a reliable manner. These models have been found valuable to improve our understanding of disc degeneration yet found limited by the adverse chemical impact of chemonucleolytic agents on the disc tissue and cells [172, 173] or by means of "artificial perturbation" produced by puncture or stabbing which does not accurately simulate the degeneration process observed clinically. Another significant limitation of these models is that the mode of mechanical load mostly investigated is the compressive load [156-166, 169, 174]. In contrast, our animal model focused on a shear load based on the hypothesis that the shear load is an abnormal spinal load whereas an axial compression is the normal load. There are a number of studies that support this hypothesis. Patwardhan and his colleagues [175-178] suggested the follower compressive load as a normal physiologic spinal load where the ligamentous spinal column can support a large compressive load without a substantial

loss of its flexibility. Recent analytical studies [179] also demonstrated that the spinal muscles may function in a coordinated fashion to maintain the follower load in the spine, and that it may experience forces in other directions, such as anteroposterior and/or mediolateral abnormal shear forces if the spine loses its stability resulting in the disruption of the follower load path. On such a theoretical basis, our animal model was the first to demonstrate the adverse effect of shear load resulting in IVD degeneration and/or spinal instability. It is also noteworthy that the degenerative changes resulted from the application of a shear stress (0.33 MPa with the average cross-sectional area of the rat L5-L6 disc computed as 12 mm^2) in this study that is much less than a critical compressive stress (>0.8 MPa) found in another study [158]. The degenerative changes in response to a lower stress observed in this study indicate the greater detrimental role of shear stress as compared to compressive stress, and this, in turn, would be more evidence supporting the concept of a follower load which suggests that shear load may be an abnormal spinal load.

In addition to disc degeneration, pain behavior of the animals in both groups was investigated by measuring MWT using von Frey filaments. This behavioral testing has been frequently used in numerous pain studies [37, 180-184] where its principle is to detect pain sensation evoked at sites adjacent to or remote from an injury site termed secondary hyperalgesia [180, 181]. Those studies include the spinal nerve injury or a herniated disc lesion in which hyperalgesia of the hindpaw to mechanical stimuli was found to occur [37, 182, 183]. The results of this study showed a significant decrease in MWT values in the experimental animals in contrast to no changes in the controls. The decrease started at 1 week and remained at the pain level until the longest duration of 8

weeks tested. It is generally believed that secondary hyperalgesia is a consequence of central sensitization in which the neuronal excitability in the spinal cord and brain is enhanced by a peripheral injury [185]. The prolonged stimulation of C-fiber nociceptors triggers calcium entry and post-translational changes in the signaling pathways of the cells surrounding the secondary site, thus serving as an indicator of pain on the primary site [186]. This implies that entering nociceptive activity from an area of primary hyperalgesia is necessary for the development and maintenance of secondary hyperalgesia in the central nervous system, eventually leading to the development of chronic pain. In this context, the secondary hyperalgesia observed in this study seemed to occur most likely by certain prolonged noxious stimuli created by the shear load in the vicinity of the L5-L6 segment.

In order to test if the degenerated discs in the shear loaded group would be a primary nociception site, the intervertebral disc and end plate sections were stained by indirect immunoflourescence for 1) the angiogenesis marker, VEGF; 2) the nociceptor marker, substance P; and 3) the inflammatory markers interleukins IL-1β and TNF-α. Positive staining of VEGF were found in the outer annulus area only in all groups, while no positive results of substance P, IL-1β, and TNF-α were observed. These results indicate no signs of neovascularization, nerve ingrowth, and inflammation in the discs and endplate regions regardless of the disc degeneration. Therefore, given the moderate degeneration present in our study, it is reasonable that the expression of this cytokine was not detected. Indirect immunofluorescence was also used to detect the presence of Pcreb and c-Fos in the dorsal horn of the spinal cord. Little to no positive staining was found in the dorsal horn area of the spinal cord to confirm pain. One of the main reasons for these

occurrences in the spinal cord as well as the IVD could be the timing of the testing itself. The expression of the cytokines and the proteins used are transient [93, 142-144, 187] and we may not have captured the spinal cord or the IVD at the time of expression. Nevertheless, the results of our immunohistochemical analyses suggest that the degenerated discs would not be the primary source of the mechanical hyperalgesia observed in von-Frey tests. Two logical locations for the observed hyperalgesia are the facet joints or the ligaments surrounding the surgical site. Furthermore, a substantially thick cartilage end plate remains in the rat discs during degeneration and may prevent the neovascularization and nerve ingrowth into the middle of the discs which was found in human degenerated painful discs obtained from the surgery in previous studies [145, 188-191]. This indicates that the rat may not be an ideal animal for the study of discogenic pain.

Despite of the aforementioned unique advantages of our animal model, this study also has several potential limitations. The first limitation is that the shear load was applied only in a static manner. The spine experiences a significant level of dynamic stress, and several studies demonstrate that cells respond differently to static and dynamic loading [192, 193]. Therefore, whether dynamic stresses have any additive and/or synergistic effect on the response of the disc should be a subject of future investigations. Another limitation is the technical difficulty in controlling the direction of the shear load as it was almost impossible to achieve an exact alignment of the SAUs, particularly with no inter-specimen variations. The third limitation was found in the difficulty of maintaining the magnitude of the load consistently over the follow-up period. The magnitude of the shear load was determined by the compressed length of the spring in the

ESLD, however, it varied *in vivo* due to the several reasons such as the viscoelastic properties of the IVD, constant movement and rapid growth of the animals. Thus, *in vitro* experiment is recommended for better understanding of the effect of mechanical loads on degenerative changes where control of mechanical stimulus is much easier. For such studies, the use of an *in vitro* disc culture system as demonstrated by Lim et al. [194] would be a viable option paralleled with the *in vivo* studies. The fourth limitation, as mentioned previously, is that we tested the samples for chronic expression of the cytokines, neuropeptides, and proteins. Acute inflammatory or pain events may have been missed due to the transient expression of these substances. Spinal cords and IVDs were collected at the end of 4- and 8- weeks; therefore, early expression of these substances would not have been detected. In order to confirm the presence of the cytokines and proteins, it might be necessary to test the samples at various time intervals throughout the study.

In addition to the limitations imposed by our experimental design, there were several sources of behavioral changes that could have impeded the results of this study. During the follow-up period, the caging was changed due to contamination. The new cages were smaller and forced the rats to contort into awkward positions to access food and water. This may well have caused the rat to twist/damage muscles which could manifest as the hyperalgesia seen in the pain behavior results. The animals were initially housed in a quiet lab with little to no outside distractions. During the course of the experiment, the rats were moved to another facility for reasons beyond our control. At this facility, the rats were housed on the bottom floor underneath the site of internal construction work on the levels above. The rats were prone to loud, unexpected noise

and vibrations as a result of the construction work. Rats are known to be stressed by a variety of factors including horizontal oscillations, new environments, and handling [195]. Some of the manifestations of this stress are burying, vocalization, and defecation and were observed during these experiments. One study showed that hyperalgesia in rats can be induced by emotional stress [195]. Therefore, given the aforementioned circumstances, it is quite possible that the hyperalgesia we observed during the course of this experiment could have been due to the changes in environment, caging, and/or a combination of the two.

CHAPTER 6

CONCLUSIONS, FUTURE WORK

6.1. Conclusions

Low back pain is a major problem in this country and around the world. It is the second most common reason for doctor's visits and costs the population billions of dollars per year. . Degenerative disc disease is a condition that involves back pain arising from causes other than cancer, tumor, or sciatica. Patients with this disease suffer from discogenic low back pain—back pain of unknown origin. Treatments for this condition (discogenic low back pain) are vast and do not always relieve the pain. Some of the contributing factors of discogenic low back pain are genetics, age, job satisfaction, and smoking. Mechanical stress, aging, disc degeneration, and inflammation all serve as possible causes of low back pain; however, the exact cause still remains unclear. Many studies have focused their efforts in determining the role of compressive forces in the generation of low back pain. Attention has been placed on the biochemical, structural, and mechanical property changes resulting from disc degeneration. Low back pain with sciatica has been studied intensively as well. However, not many focus on discogenic low back pain as a result of abnormal mechanical stress. To date, only one study has investigated the effects of an abnormal load to the lumbar spine in vivo.

This study was designed to enhance the previous knowledge of the effects of abnormal loading on the spine in vivo in attempt to resolve one of the causes of disc degeneration and low back pain. Studies have shown that painful degenerated discs

exhibit unusual characteristics such as nervous ingrowth into the subchondral bone, AF and NP regions. They also confirmed the ingrowth of blood vessels in conjunction with nerve innervation. Evidence of inflammation has been found in degenerate and herniated discs. For these reasons, we developed a study that would induce degeneration and possibly pain and used the knowledge of previous studies to confirm or negate our hypothesis. To accomplish this, we attached a novel shear loading device to the L5-6 rat spine to impart an abnormal shear load and then examined the IVD for signs of disc degeneration histologically as well as immunohistochemically. To confirm pain, we performed mechanical withdrawal tests and employed immunohistochemistry again to check for pain markers in the spinal cord. Grade 1 degeneration was found in the intact control and immobilization control samples in accordance with a recent study that states that normal rats young and old, have this level of degeneration. We found disc degeneration in all of our experimental animals in loaded and unloaded segments. Significant degeneration was found when the control group was compared to the 4- and 8- week loading groups. Pain behavior testing confirmed the presence of pain, however, we determined that they hyperalgesia must have been a result of pain in structures not tested. Immunohistochemical analysis revealed the presence of the blood vessels in the vertebral bodies, but showed no evidence of chronically expressed inflammatory cytokines or nociceptive neurotransmitters in the IVD. The inflammatory episode may have been acute, in contrast to the chronic levels that were tested. We checked the spinal cord for evidence of pain, however, no real evidence could be found. Cytokine and protein expression are dependent upon exposure to noxious stimuli and may occur early on in the degeneration process. we may have missed the time of expression. Given these

results, we state the following conclusions. The abnormal shear loading of the L5-6 segment caused degeneration of not only the experimental level, but adjacent ones as well. Evidence of pain was found using the mechanical withdrawal testing, but immunohistochemical analysis did not confirm chronic pain as a result of the IVD. However, immunohistochemical testing at different time points may reveal expression during early degeneration. Therefore, the primary source of hyperalgesia was not due to disc degeneration; however, the exact source of this pain behavior is unclear. In addition, we found that prolonged application of shear force in the lumbar spine might produce scoliosis as described in the Appendix.

6.2. Future Work

Great lessons have been garnered from this study. As mentioned previously, we believe that the rat may not be the ideal animal to induce degeneration with nervous ingrowth. The cartilaginous endplate in the rat IVD remained thick and relatively unchanged throughout the experiment despite degeneration of the AF and NP. It is for this reason that we feel the rabbit may be a better suited model. The rabbit IVD properties have been investigated extensively [73]. It was determined that the cells of the NP originate from the cartilaginous endplate. Therefore, the cartilaginous endplate becomes thinner as the disc degenerates providing a possible gateway for emerging nerves and blood vessels from the induction of disc degeneration. Rabbits are larger animals and would make the technical aspects of the surgery easier to handle (i.e. insertion of screws into the base of the SAU). Finally, unlike the rat, there is a definitive

characterization of the changes that occur during disc degeneration. This would enable a more histologically sound evaluation of the IVD.

APPENDIX

Thoracolumbar Scoliosis Produced by Sustained Application of Shear Force on the L5-L6 Segment: An *In-vivo* Rat Study

Idiopathic scoliosis is scoliosis of no known cause and is clinically defined as a lateral curvature of the spine that exceeds 10°. It usually affects female adolescents aged 11-17 more frequently than males and its progression is exacerbated by changes that occur during puberty. Studies have been performed in an attempt to understand its origins and have included a variety of methods to obtain the scoliotic spine. Mechanical methods such as tethering and the use of pins as well as surgical methods such as pinealectomy have produced scoliotic animals. Melatonin deficiency[196], mechanical perturbation of the spinal elements including the surrounding musculature[197], and nutritional deficiencies[198] have been named as possible factors contributing to the etiology of scoliosis. Machida et. al. have found that pinealectomized chickens developed idiopathic scoliosis as a result of decreased melatonin levels. There are conflicting results in the literature as to whether the same is true for humans[199]. Most interesting is the relation of Prune Belly Syndrome to scoliosis. In this disease, the patient suffers from poor development of abdominal muscles. One study reports that 4-36% of these patients develop scoliosis later in life [200]. We postulate that disruption in spinal muscles leads to degeneration of the disc. If this is true, then the abnormal muscle response to shear loading could be a possible source of idiopathic scoliosis. There are no biomechanical studies that sufficiently link scoliosis to adverse mechanical loading. To

clarify the origins of idiopathic scoliosis, a reliable animal model must be established to accurately pinpoint and isolate the causative factors.

We identified abnormal deformation of the spines during the harvest of the spines from the animals in the 8 week loading group and conducted radiological observations on 4 animals in the 8 week shear loading group, 4 intact animals and 6 animals in the 4 week loading group. Anterior-posterior (AP) radiographs of the animals were obtained and used to measure the Cobb's angle of the thoracolumbar spine (T8-L3) using Image J software. The mean Cobb's angle was determined from the measured data by 4 observers in a blinded manner.

As shown in Figure A1, the lateral deformation of the thoracolumbar spine resembling the scoliosis curve was developed in 4 weeks after loading and the further increase in the Cobb's angle with longer loading to 8 weeks. The mean (±SD) values of the measured Cobb's angles for each group were 4.8°(±2.5) in the intact group, 23.5°(±7.3) in the 8wk Group, and 10.3°(±2.2) in the 4wk group. The measures are charted in Figure A2. The Cobb's angle of 8 week loading group was significantly greater than that of the intact and 4 week loading groups ($p < 0.01$) while there was no significant difference between the intact and 4 week loading groups.

There is no known cause of idiopathic scoliosis. There has been a correlation of abnormal abdominal musculature to the development of scoliosis. The spinal and abdominal muscles play major roles in maintaining the spine in its correct upright orientation. It is reasonable to assume that if the muscles are sufficiently impaired, the spine might develop abnormally. In this study, we saw that the spine did curve

abnormally with the addition of the abnormal load. Therefore, the use of this model to induce scoliosis may be a worthwhile endeavor.

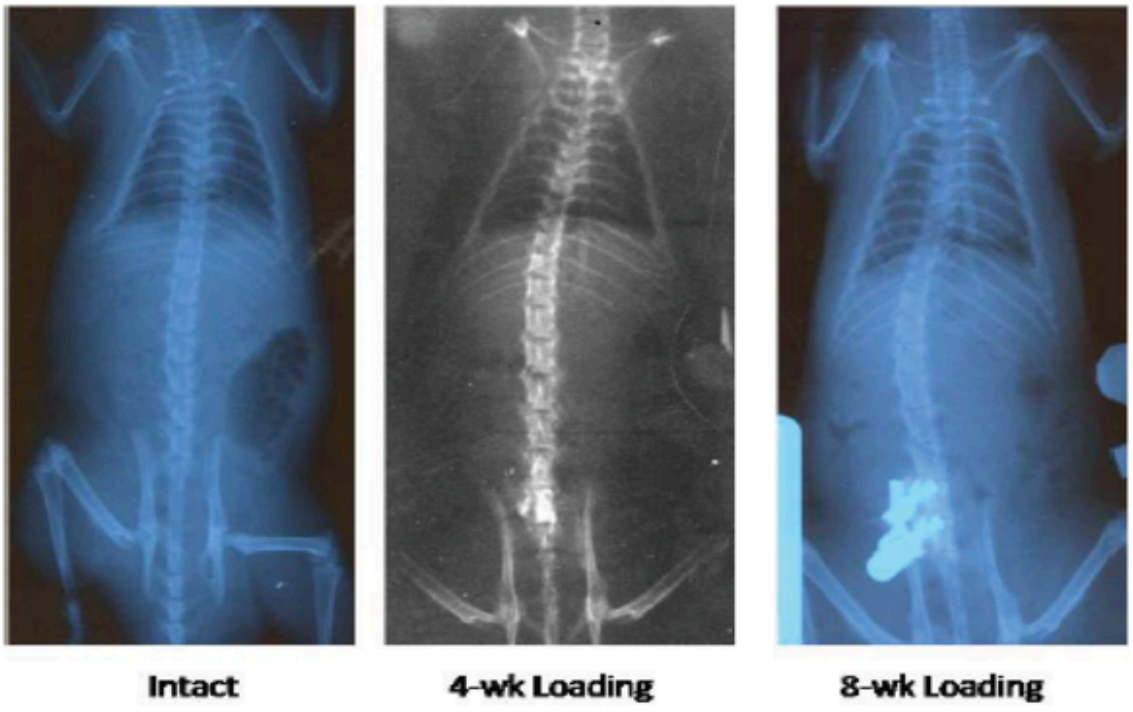

Figure A-1: Radiographs of the experimental rats. A relatively straight intact control spine is shown in contrast to the lateral curvatures seen in the 4- and 8- week loading groups.

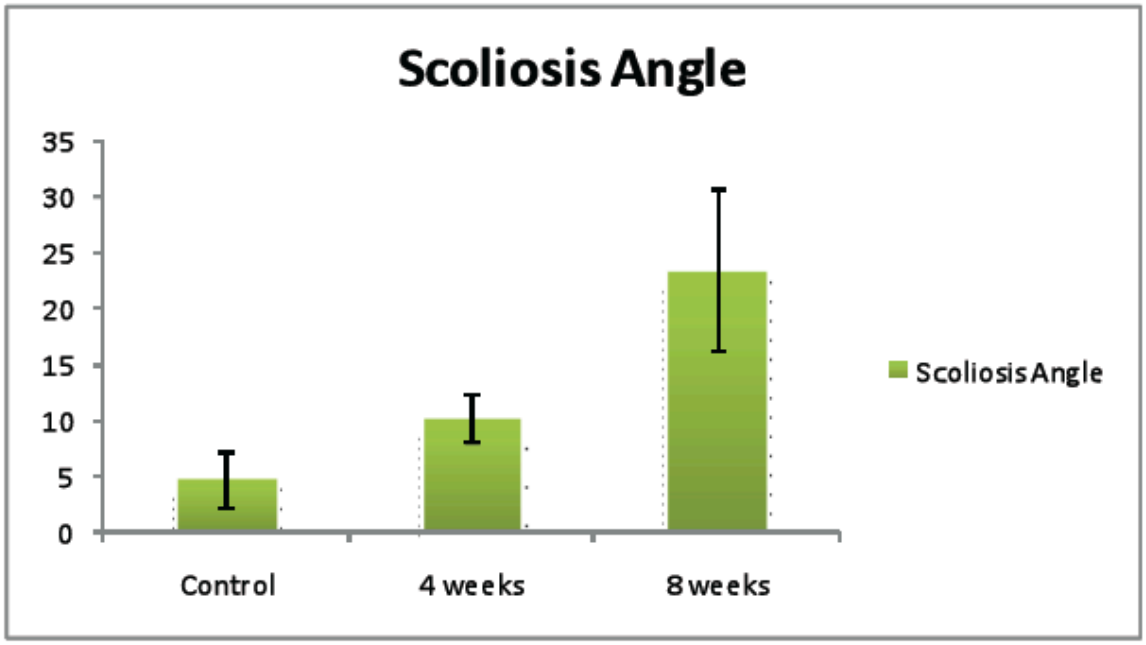

Figure A- 2: Graph comparing the intact and experimental Cobb's angle measurements.

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