The effect of environment on post surgical overall well-being and pain sensitivity in an animal model

https://hdl.handle.net/2144/14941

Boston University
THE EFFECT OF ENVIRONMENT ON POST SURGICAL OVERALL WELL-BEING AND PAIN SENSITIVITY IN AN ANIMAL MODEL

by

ARCHANA REDDY

B.S., University of South Florida, 2012

Submitted in partial fulfillment of the requirements for the degree of Master of Science 2014
First Reader
Theresa A. Davies, Ph.D.
Director, M.S. in Oral Health Sciences Program
Adjunct Assistant Professor of Biochemistry

Second Reader
Christine Sieberg, Ph.D.
Attending Psychologist, Pain Treatment Service,
Department of Anesthesia - Boston Children's Hospital
Assistant Professor, Department of Psychiatry - Harvard Medical School
THE EFFECT OF ENVIRONMENT ON POST SURGICAL OVERALL WELL-BEING AND PAIN SENSITIVITY IN AN ANIMAL MODEL

ARCHANA REDDY

ABSTRACT

With chronic post surgical pain affecting up to one third of patients undergoing surgeries and the price of treatment being astoundingly high there has been a transition in research to investigate and identify risk factors. Through identification of risk factors new preventative measures can be taken to ensure better surgical outcomes. The role that psychosocial factors can play in the development of chronic post surgical pain has long been recognized yet its mechanisms are still unknown. We aim to investigate how environment can play a direct role in pain perception and sensitivity. We used a Chronic Mild Stress (CMS) paradigm to induce depression in 10 adult male mice, we used 10 control mice who were left in standard opti cages, and 10 enriched mice who were placed in large enrichment cages. CMS mice were exposed to a series of stressors and all mice underwent spared nerve injury surgery. During spared nerve injury the common peroneal and tibial branches of the sciatic nerve were severed while the sural branch was left intact. Overall well-being and pain threshold of mice were tested via Von Frey, Hot Plate, Heat Place Preference, Dynamic Weight Bearing, Hole Board, and Social Interaction. It was found that CMS mice experienced thermal hyperalgesia yet normal thermal threshold sensation. CMS mice also spent less time interacting with novel mice in social interaction, and less amount of time exploring the center of the hole board arena than
control or enriched mice. While Von Frey results did not change over the course of the experiment, dynamic weight bearing results indicated spared nerve injury surgery was successful and produced chronic pain. Results indicate that environment plays a role in thermal pain perception and CMS affected overall well being of mice as CMS mice exhibited more timid and anxious behavior.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE</td>
<td>i</td>
</tr>
<tr>
<td>COPYRIGHT PAGE</td>
<td>ii</td>
</tr>
<tr>
<td>READER APPROVAL PAGE</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iv</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xiii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Acute to Chronic pain</td>
<td>1</td>
</tr>
<tr>
<td>Types of Chronic Pain</td>
<td>5</td>
</tr>
<tr>
<td>Risk Factors</td>
<td>6</td>
</tr>
<tr>
<td>Pre-Operative Pain</td>
<td>7</td>
</tr>
<tr>
<td>Age</td>
<td>7</td>
</tr>
<tr>
<td>Genetics</td>
<td>7</td>
</tr>
<tr>
<td>Intra-Surgical Factors</td>
<td>8</td>
</tr>
<tr>
<td>Acute Post-Operative Pain</td>
<td>8</td>
</tr>
<tr>
<td>Psychological</td>
<td>8</td>
</tr>
</tbody>
</table>
Thermal Place Preference ................................................................. 28
Hot Plate ......................................................................................... 28
Dynamic Weight Bearing ............................................................... 28
Von Frey Test .................................................................................. 29
Spared Nerve Injury ........................................................................ 29

RESULTS ......................................................................................... 32

PRE-SNI DATA .................................................................................. 32
Hot Plate ......................................................................................... 32
Dynamic Heat Place Preference ..................................................... 33
Holeboard Experiment ................................................................. 34
Von Frey ......................................................................................... 37

Social Interaction ........................................................................... 38

POST- SNI DATA ............................................................................... 40
Dynamic Weight Bearing .............................................................. 40
Post SNI Von Frey ........................................................................... 42

DISCUSSION .................................................................................... 42
Hot Plate ......................................................................................... 42
Dynamic Heat Place Preference ..................................................... 43
Holeboard Experiment ................................................................. 43
Social Interaction ........................................................................... 44
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Characteristics of Neuropathic Pain</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Mild Stressors Used on Mice</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>Schedule of Unpredictable Chronic Mild Stress model and Experiments along with a key</td>
<td>20</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mechanism Outline of Development of Post-Surgical Neuropathic Pain</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Diagrams illustrating the physiological connection between depression and pain pathways in the body</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>An overview of factors investigated for the involvement in development in chronic post-surgical pain</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>Schematic diagram of the sciatic nerve and its three branches in the paw of a rodent</td>
<td>23</td>
</tr>
<tr>
<td>5</td>
<td>Hot Plate Response times of mice at 52°C</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>Average amount of time spent on plate at a given temperature of enriched, control, and chronic mild stress mice.</td>
<td>25</td>
</tr>
<tr>
<td>7</td>
<td>A bar chart showing ratio of distance travelled in center:distance traveled in periphery of holeboard experiment</td>
<td>26</td>
</tr>
<tr>
<td>8</td>
<td>A bar chart showing a ratio of time spent in center: time spent in periphery of mice in the holeboard experiment</td>
<td>27</td>
</tr>
<tr>
<td>9</td>
<td>Percentage of total time was spent in the center of the holeboard</td>
<td>27</td>
</tr>
<tr>
<td>Page</td>
<td>Section Description</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Baseline Von Frey Values Pre SNI</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Habituation times in empty and novel mouse chambers of mice pre- and post-SNI surgery</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Interaction times in empty and novel mouse chambers of mice pre- and post-SNI surgery</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Weight bearing on left and right hind paws on days 8 and 16 post SNI</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Weight distribution ratios between left and right hind paws on days 8 and 12 post SNI</td>
<td></td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

AMPA ........................................ $\alpha$-amino-3-hydroxy-5- methyl-4-isoazolepropionic acid
CBT .......................................................... cognitive-behavioral therapy
CMS .............................................................. Chronic Mild Stress
CNS .............................................................. central nervous system
COX-1 ......................................................... cyclooxygenase 1
COX-2 ......................................................... cyclooxygenase 2
CPSP ............................................................ Chronic Post-Surgical Pain
GABA ......................................................... gamma-aminobutyric acid
IB4 lectin ........................................................ isolectin Griffonia simplicifolia I-B4
Mg2+ .............................................................. magnesium
mGLuR ......................................................... G-protein-coupled metabotropic
MMPI .......................................................... Minnesota Multiphasic Personality Inventory
NDMA ......................................................... N-methyl-D-aspartic acid
NSAIDs ........................................................ non-steroidal anti-inflammatory drugs
PNS ............................................................... peripheral nervous system
ROM ............................................................. range of motion
SNI ............................................................... spared nerve injury
TCAs .......................................................... Tricyclic antidepressants
TENS ........................................................ Transcutaneous Electrical Stimulation
TNF ............................................................. tumor necrosis factor
UCMS....................................................... Unpredictable Chronic Mild Stress

WDR................................................................. Wide Dynamic Range
INTRODUCTION

With up to one third of patients who undergo common surgical procedures reporting persistent or intermittent pain one year postoperatively it is no wonder there is an increase in research on chronic post-surgical pain (CPSP) and factors contributing to it\textsuperscript{1}. Of those patients who experience chronic pain after surgery about 2-10\% of those patients report it being severe\textsuperscript{2}. CPSP is not only difficult but costly to treat, at a cost of $635 billion per year and a reduced quality of life\textsuperscript{3}. By identifying and confirming factors involved with CPSP preventative measures can be taken with at-risk patients resulting in a reduced incidence of chronic post-surgical pain. This impact would be profound considering the number of surgical procedures that occur annually.

While there may not be a single standard definition of post-surgical chronic pain Macrae and Davies proposed four specific criteria that should be met in order for chronic pain to be classified as post-surgical: First, the pain must develop after a surgical procedure. Secondly, the pain is of at least two months duration. Additionally, other causes for the pain have been excluded and finally, the possibility that the pain is from a pre-existing condition has been excluded\textsuperscript{1}.

Acute to Chronic pain

In the case of post-surgical chronic pain it is most common that the chronic pain originated from post-surgical acute pain. An incision can cause sensitization of the central nervous system via a series of chemical reactions that begin at the site of the surgery\textsuperscript{4}. 

1
During surgery activation of noicceptors (peripheral nerves that sense harmful chemical, mechanical, and thermal stimuli and relay information to the central nervous system (CNS)) carries afferent signals of injurious stimuli to the central nervous system (cerebral cortex). There are two main types of noicceptor, they include the A-delta fibers which are myelinated and of medium diameter. A-delta fibers are rapid conducting fibers and are activated in response to rapid, sharp pain (for example heat exposure). The other type of noicceptors is the C fibers. C fibers are unmyelinated and are of small diameter making them slow response fibers, activated in response to delayed and dull pain (due to tissue damage or medical disease). These noicceptors can be sensitized, making them more responsive stimuli; this can be such an extreme sensitization that harmless stimuli can be perceived as painful. Signals from noicceptors synapse onto the dorsal horn where they are modified. Wide Dynamic Range (WDR) neurons in the dorsal horn receive information from harmless stimuli as well as from noicceptors. However, repeated input from noicceptors can cause sensitization of these WDR neurons and lower their threshold. This sensitization contributes to the “wind up” phenomenon.

Figure 1 illustrates the steps involved in the case of a severed or injured nerve.
Figure 1. Mechanism Outline of Development of Post-Surgical Neuropathic Pain (Amended from Chronic Post-Surgical Pain⁶).

1. There will be an inflammatory response in which the distal end of a severed nerve will degenerate and be engulfed by inflammatory cells. A number of signal molecules will be released which induce pain, such as tumor necrosis factor (TNF), these molecules act to increase surface activity of neurons. In the spinal cord macrophage cells, otherwise known as microglia cells, are activated and induce hypersensitivity in neurons of the dorsal horn via a number of molecules. These molecules act by changing gene expression which leads to central sensitization.
2. This hypersensitivity is amplified in the dorsal horn via loss of inhibitory neurons and microglial activation.
3. The transmission in the spinal cord is modulated by the descending controls in the brainstem.
4. Altered mood, behavior, and autonomic responses are seen due to the limbic system and hypothalamus.
5. Sensations of pain are generated by the cortex and the types of sensations experienced are determined by cultural input, past experience, and expectations.
6. It is also thought that an individual’s genomic DNA can play a role in whether a patient develops CPSP or not.
7. C fibers are found to have higher threshold than A fibers in normal sensitivity states. However post surgery prolonged firing of C-fibers can lead to high release of glutamate.

There are three types of ionotropic glutamate receptors that are present post-synaptically; the N-methyl-D-aspartic acid (NDMA), the α-amino-3-hydroxy-5- methyl-4-isoxazolepropionic acid (AMPA), and the G-protein-coupled metabotropic (mGLuR) receptors. In the case of chronic pain when there is prolonged stimulus from C-fibers a process known as wind up occurs via activation of NDMA receptors. During normal sensitivity states NDMA magnesium (Mg2+) channels are blocked, however during development of chronic pain when C-fibers are continuously stimulating the NDMA receptors substance P and CGRP (from C fibers) facilitate the unblocking of these Mg2+ channels. These activated NDMA channels now play a role in secondary hyperalgesia and lead to changes in second order neurons that play a role in pathogenesis of chronic pain.
There is such a wide range of changes seen in the nervous system with acute and chronic pain with both peripheral noiciceptors and central nervous system changes happening. Therefore it can often be difficult to treat post surgical chronic pain.

Types of Chronic Pain
While there are psychological factors that contribute to the development is CPSP, there are a number of physical factors that may contribute to it as well. Kehlet et al assert the idea that chronic pain is a result of persistent inflammation or neuropathic pain due to nerve injury during surgery².

When noiciceptors are activated pain is the resulting feeling. This occurs during surgery (due to incision etc). This type of pain experienced is called noiciceptive pain. When tissue injury occurs the body produces an inflammatory response, in this response a number of sensitizing inflammatory mediators are released that lead to increased pain sensitivity in the noiciceptors that innervate the damaged tissue. Because of this, increased pain sensitivity stimuli that do not usually elicit pain will do so. Unless there is nerve damage, inflammatory pain is the cause of post-surgical chronic pain. If the inflammatory response of the body continues, so will the pain².

Neuropathic pain is the pain experienced when there has been a nerve injury or damage/alteration to a part of the spinal cord or brain involved in sensory perception. A feature that distinguishes neuropathic pain from other types of pain is the experience of sensory loss (such as loss of touch, temperature, or pressure with damage to afferent nerves) along with hypersensitivity, spontaneous pain, and pain induced via normal
stimuli. These patients can experience a greater intensity of pain in response to harmful stimuli or pain that lasts longer than the period of time the stimulus is applied. This increased sensitivity and abnormal sensory experience spreads beyond the area of the damaged nerve\(^2\).

In order to treat and prevent such chronic pain physicians must distinguish when chronic pain is inflammatory versus neuropathic. In order for pain to be established as neuropathic it must meet a number of factors (Table 1).

**Table 1: Characteristics of Neuropathic Pain.** (Table amended from Persistent postsurgical pain: risk factors and prevention\(^2\))

- Pain in a neuroanatomically defined area—ie, corresponding to a peripheral or central innervation territory
- A history of relevant disease or lesion in the nervous system, which is temporally related to development of pain
- Partial or complete sensory loss in all or part of the painful area
- Confirmation of a lesion or disease by a specific test—eg, surgical evidence, imaging, clinical neurophysiology, biopsy

**Risk Factors**

There are many risk factors that have been identified for CPSP; they can occur pre-, intra-, and postoperatively. Some of the risk factors thought to contribute to the
development of CPSP include preoperative pain, age, genetic predisposition, intraoperative nerve damage/handling, and post-operative acute pain management\textsuperscript{8}.

How these factors increase probability of postoperative chronic pain is constantly being studied and the information on them expanding.

\textit{Pre-Operative Pain}

It was found that chronic pain after surgery was predicted by pre-surgical at or near the site of operation\textsuperscript{9,10}.

\textit{Age}

In the case of age, younger patients were found to be more likely to develop CPSP with the probability of developing CPSP decreasing with each year of age in the case of breast cancer. However, the mechanism of this still is not known\textsuperscript{11}.

\textit{Genetics}

The idea that genetic factors act as a risk factor for CPSP are grouped with environmental factors. It is fair to include these as there is a wide variety of pain sensitivity in humans and animal. Even in people undergoing similar surgeries and sustaining similar nerve/tissue damage and same concentration of nerves in an area only some develop chronic pain after surgery. It is thought that single nucleotide polymorphisms in the gene coding for the catecho- O- methyl-transferase enzyme\textsuperscript{12} and variability in the expression of enzymes responsible for neurotransmitter synthesis in ganglions may be responsible for chronic pain development in some surgeries\textsuperscript{13}. 


Intra-Surgical Factors

During surgery many nerves are susceptible to damage (being stapled to mesh etc, severed, entrapment in scar tissue, crushed, stretched, or chronic inflammation due to infection or foreign material), this nerve damage may lead to chronic pain development\textsuperscript{1}. Longer surgeries (those over 3 hrs) may also result in a greater chance of developing CPSP\textsuperscript{14}.

Acute Post-Operative Pain

In many studies it has been found that patients experiencing higher levels of acute post-operative pain were more likely to develop chronic pain weeks and months after surgery\textsuperscript{1}. There have been many studies that express that adequate pain treatment in the acute phase is important and can reduce the chances of chronic pain\textsuperscript{6}.

Psychological

Among the factors thought to contribute to CPSP are psychological factors, an area that this research will focus on. It seems that research has now shifted to the idea that pain is now the result of not only biological but psychological factors also. Factors such as pre-surgical anxiety, depression, fear, catastrophizing, fear of pain and dispositional pessimism are all considered risk factors for higher post surgical pain and development of chronic pain\textsuperscript{1}.

According to Block et al psychological factors can contribute to the outcome of spine surgery itself\textsuperscript{15} and Spengler and his colleagues found that the Minnesota Multiphasic Personality Inventory (MMPI) Hypochondriasis and Hysteria scales better predicted
clinical outcome than physical examination results\textsuperscript{16}. Block also found that patients who were psychologically at risk for poor post surgical results reported a lower reduction in pain post surgically than those who were not psychologically at risk. They also discovered that in patients undergoing diskography on the back, those who reported pain even upon injection of a non disrupted disk had higher scores on the MPPI Hypochondriasis and Hysteria scales\textsuperscript{15}.

In a study performed by Brander and colleagues patients who experienced more severe pain were also patients who had been diagnosed with the most severe pre-operative depression\textsuperscript{17}. In a study on patients undergoing laparoscopic cholecystectomy it was found that patients who reported persistent pain post surgery also reported having used or were currently using more psychotrophic drugs (antidepressants etc.), however those who hadn’t been using psychotrophic drugs reported better post surgical outcomes\textsuperscript{18}.

In a study on patients undergoing abdominal surgery a State-Trait Anxiety Inventory was used to measure anxiety (state and trait anxiety). State anxiety is situational anxiety and is transient, while trait anxiety is a steady level of fearfulness that occurs as a part of personality. It was found that trait anxiety was a predictor for higher intensity pain, and those who were found to have high trait anxiety were much more sensitive to stimuli\textsuperscript{19}.

Catastrophizing has been found to be a strong predictor of post surgical pain and poor outcome of surgery. It was found in multiple studies that a correlation between catastrophizing and pain intensity exists\textsuperscript{20,21}. 
Depression and Pain

The relationship between pain and depression is apparent in the circuitry of the nervous system\textsuperscript{22}. It has been found in many studies that depressed patients tend to score higher pain scores than those who are not\textsuperscript{11}. The serotonin and nor epinephrine descending pathways are important for suppressing attention to minor discomforts and some painful stimuli. These neurotransmitters play an important role in regulation of emotion. A malfunction in serotonin and norepinephrine levels will also have a malfunction in these pathways and can result in increase of pain intensity along with depressive symptoms such as sadness, anxiety, hopelessness, or inability to cope with stress. Therefore some patients with depression can report lower pain threshold and chronic pain symptoms\textsuperscript{23}.

The descending pathways from the brain stem flow into the dorsal horn where pain modulation of incoming stimuli from afferent neurons takes place. Modulation involves a number of substances and neurotransmitters. The two main nociceptive afferent pathways that synapse onto the dorsal horn include one which uses substance P among other substances and the other uses IB4 lectin (isolectin Griffonia simplicifolia I-B4). Glutamate is a neurotransmitter than excited both these pathways and has therefore been the target of some drug development research\textsuperscript{24}. As pain and depression are physiologically linked it is possible that depression can be a risk factor for the development of chronic pain, allodynia, and hypersensitivity post surgery. Depressed patients may have greater difficulty coping with post operative pain\textsuperscript{24}. 
Figure 2 shows the parts of the brain affected by serotonin and norepinephrine as irregular levels of either neurotransmitter leads to depression here. Figure 2 also shows descending modulatory pain pathways of norepinephrine and serotonin.

Figure 2. Diagrams illustrating the physiological connection between depression and pain pathways in the body. In Figure 2 it is seen that serotonin and norepinephrine in the brain that is responsible for emotional stability also have descending pathways that play a role in pain perception (Figure Amended from When Women Hurt in Mind and Body: Managing Depression and Physical Symptoms25)
Figure 3. An overview of factors investigated for the involvement in development in chronic post-surgical pain. Figure 3 shows a number of investigated factors that may be involved with the development of Chronic post surgical pain and the role they may play. (Figure Amended from Chronic Post-Surgical Pain')

Preventative Measures
As treatment can be difficult and expensive there has been a shift in attention towards prevention. The risk of damage to nerves is common in many surgeries that have a frequent outcome to post surgical chronic pain. Therefore, there is a great value placed on alternative surgical techniques, better precision, or avoiding surgery altogether. Examples of changes in surgical techniques that may help in the prevention of chronic pain include sparing the intercostal brachial nerve during mastectomies, using a mesh in the repair of
inguinal hernias\textsuperscript{27}, and using intracostal suture could avoid direct nerve compression in a thoracotomy\textsuperscript{28}.

It has been shown in many studies that patients with higher presurgical anxiety, depressive symptoms, or emotional distress tend to experience higher post surgical pain and poorer surgical outcomes\textsuperscript{6}. Therefore psychological preparation and stress relieving methods for patients may have a beneficial outcome in reducing post operative pain\textsuperscript{6}.

Pre-emptive and multi modal analgesia has been shown to reduce acute pain following tissue injury that occurs during surgery. The idea is that the use of anesthetics prior to surgery will prevent the alteration of central sensory processing (as seen with neuronal plasticity) that leads to increased pain sensitivity and allodynia post surgery\textsuperscript{6}. It was found in a number of studies on 3261 patients that preemptive analgesia showed a beneficial effect in lower post operative pain in some analgesic regimens. These benefits were most pronounced following epidural analgesia, local wound infiltrations, and systemic non-steroidal anti-inflammatory drugs (NSAIDs)\textsuperscript{29,30}.

The research on preventive analgesia is also expanding, the results of which still seem unclear. In studies performed by McCartney et al in which magnesium was used, it was reported that ketamine and dextrometorphan (N-methyl-D-aspartate receptor antagonists) had no significant positive effect\textsuperscript{31}. However, in a study performed by Lavand’homme et al it was found that the use of epidural analgesia along with systemic ketamine were effective in containing hypersensitivity surrounding incision to a smaller area and reducing late residual pain\textsuperscript{32}. It was also found that the use of epidural analgesia before
surgery and continued use post surgery reduces incidence of post surgical pain in patients having undergone laparotamy or thoracotamy. A reduction in incidence of post surgical chronic pain was also seen in the use of a paravertebral pre and post surgery in patients undergoing breast and thoracic surgery.

In an analysis performed by Akkaya and Ozkan evaluating 12 studies and 896 patients it was determined that the use of orally administered gabapentin shortly before surgery was effective in providing relief from pain. Gabapentin is an anticonvulsant that acts by binding and inhibiting pre-synaptics voltage dependent Ca$^{2+}$ channels. Therefore the release of neurotransmitters, such as glutamate, is inhibited from the ends afferent sensory neurons that transmit pain information to neurons in the spinal cord.

In some studies the effects of gabapentin on chronic neuropathic pain and chronic use to oral gabapentin was found to produce a reduction in the effects of central sensitization. In a number of studies gabapentin was also shown to have synergistic effects on pain when combined with other analgesics. Oral gabapentin seems to be effective drug in reducing hypersensitivity and pain post surgery and therefore may be a good preemptive treatment for post surgical chronic pain.

**Experimental Procedures Used**

**Unpredictable Chronic Mild Stress Model**

In this experiment we used an animal model of depression called the Unpredictable Chronic Mild Stress (UCMS) model. The model, introduced by Willner et al, is said to induce anhedonia after several weeks. Anhedonia is measured by a reduced intake or
preference for sucrose solution by mice; this is a characteristic of depression. In this model animals are subjected to at least one of a number of mild stressors every day for a period of a few weeks. The stressor chosen on a given day is varied throughout the period of time. The effect of the UCMS model was measured by social interaction experiments and holeboard experiments.\textsuperscript{35}

**Holeboard**

The holeboard experiment, introduced by Boissier and Simon, consists of a holeboard apparatus which is an area in which there are holes in the floor that an animal may place its head into when exploring.\textsuperscript{36} The number of times an animal dips its head into a hole and the duration of it is measured; it is assumed that higher frequency head dipping and longer duration of head dipping is associated with animals that are more likely to explore and are less timid. Animals that avoid head dipping are thought to be more timid and highly anxious.\textsuperscript{37}

**Social Interaction**

Depression is characterized by a disruption in social behavior. The apparatus is a three chamber box in which the mouse can choose to spend time in one or two of the empty chambers or to indirectly interact with an unfamiliar mouse. It is expected that control mice will spend more time interacting with a novel mouse than in either of the empty compartments, and a mouse with impaired social behavior (as that which happens in depression) will spend less time interacting with a novel mouse than a control mouse.\textsuperscript{38}
Heat Place Preference

The sensitivity of the mice to pain was measured by the hot plate preference test and the Von Frey test. In the hot place preference paradigm a mouse is placed on two plates that fluctuate between two temperatures\(^39\). The amount of time a mouse spends on a given plate at a specific temperature is measured and graphed to show its preference for temperature.

Von Frey

Von Frey is used to measure pain threshold. Developed by Von Frey, it uses hairs applied to the hind paw of the animal. The fibers increase in mass and therefore increase with pressure applied. A positive response to a fiber is measured when the animal quickly withdraws its paw\(^40\). There is an expected reduction in pain threshold with hypersensitivity.

Dynamic Weight Bearing

Dynamic weight bearing is a method for measuring mechanical hypersensitivity. It allows for the measurement of weight placed on each individual paw. A mouse is allowed to roam freely within the box and the results are measured via pressure transducers\(^41\). This experiment will be used to measure weight bearing post surgery to confirm injury.
Current Treatment

Pharmacological Approaches

There are a vast number of approaches to treating post surgical chronic pain. Many approaches to treating chronic pain include the persistent use of many pharmacological drugs used to treat post operative acute pain.

The first line of treatment usually begins with nonopioid drugs, such as acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs act by inhibiting the enzymes cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2); these enzymes are important for the production of prostaglandins with COX-2 being the form expressed in inflammatory cells. However, long term use of NSAIDs poses a risk of developing gastric and upper duodenal ulcers along with gastrointestinal bleeding, increased bleeding time, increased risk for renal toxicity, and an increase in chance of myocardial infarction, stroke, and raise in blood pressure\textsuperscript{42}.

The chronic use of opioids may also be used in pain management. In response to painful stimuli the body releases opioid proteins which are important in inhibiting synaptic transmission. This is the body’s natural defense against in pain modulating. Opioid receptors are found throughout the body (not just in the brain) and opioids act by inhibiting transmission of pain information and by increasing the activity of cells in the spinal cord that are important in descending pain inhibition. Opioid receptors are G-protein coupled receptors and may result in the closing of pre-synaptic voltage gated calcium channels. Opioid drugs act as receptor agonists. Some opioids may have two or
more ways of conferring analgesic effect such as NDMA antagonist activity. However, opioids pose the threat of dependency and addiction. Tolerance is also a problem as higher and higher doses of opioids are needed to produce the same level of pain relief\textsuperscript{42}.

Interestingly, anticonvulsants and antidepressants have also been used in the treatment of chronic pain. Analgesics have been used in the treatment of neuropathic pain as they are able to reduce the excitability of neurons. The most commonly studied are gabapentin, pregabalin and carbamazepine. Gabapentin is structurally similar to gamma-aminobutyric acid (GABA) and binds to voltage dependent calcium channels therefore inhibiting release of neurotransmitters. Pregabalin acts in a similar way, binding to voltage gated calcium channels, preventing their ability to open affect release of neurotransmitter. Carbamazepine acts by blocking sodium channels on neurons, therefore blocking the initiation of action potentials and repeated discharges of neurons\textsuperscript{42}.

Due to the close relationship of depression and chronic pain in the circuitry of the brain and their relationship with the serotonin and norepinephrine pathways, many antidepressants can be used in the treatment of chronic pain. Tricyclic antidepressants (TCAs) have been shown to relieve allodynia. Antidepressants act by inhibiting the reuptake of serotonin and norepinephrine, enhancing the effect these amines have on suppressing pain. The full mechanisms of the analgesic effects of antidepressants are not yet know. The most common side effects of TCAs include sedation, weight gain, dry mouth, constipation, nausea, diarrhea, and orthostatic hypotension which may lead to faints or dizzy spells\textsuperscript{42}.
Since much pain is caused by afferent nerve excitement in peripheral areas the use of topical agents has become common in pain management. Topical NSAIDs have been found to be efficacious in relieving pain. Capsaicin, a substance derived from chilli peppers produces its analgesic effects by reducing the amount of substance P and other pain neuro-peptides in the area applied. A lidocaine patch as well as topical TCAs and ketaminines may be used\(^\text{42}\).

**Nonpharmacologic Approaches**

Many pharmacological drugs do not provide enough relief and a number of other factors may be contributing to the chronic pain, therefore other approaches to pain management or treatment have been used.

It is apparent in many studies previously discussed that psychological factors may play a role in post surgical chronic pain development. Therefore an approach in pain management is cognitive-behavioral therapy (CBT). During this therapy patients can learn how their views and thoughts can affect their pain symptoms. Patients learn to change thoughts and behaviors\(^\text{43,44}\).

Physical medicine approaches are focused on restoring range of motion (ROM) via occupational or physical therapies\(^\text{45}\).

Neuromodulation may be another approach to pain treatment as well. TENS (Transcutaneous Electrical Stimulation) involves applying electrical currents to the skin. TENS produces paraesthesia in the area under electrodes\(^\text{46}\). Spinal cord stimulation has also been used, as well as deep brain stimulation\(^\text{47}\). In deep brain stimulation
neuromodulation of the brain is used to achieve analgesia; motor cortex stimulation has been found to be efficacious in relieving pain.\textsuperscript{47}

As discussed, there are many approaches to treatment of CPSP. However the development of CPSP includes a number of complex physiological changes that occur in not only the peripheral nervous system (PNS) but in the CNS also; this makes treatment of the disease very difficult and many of these options fail to alleviate symptoms. Psychological approaches may also play a role in the development of chronic pain postoperatively and therefore pharmacological approaches may not be enough to relieve pain. Treatment can also be highly expensive with chronic use of many drugs and invasive procedures. Therefore identification of risk factors is highly important and preemptive approaches are beneficial.
AIMS AND IMPORTANCE

While there is a vast majority of treatment options available to patients with CPSP there is still a lack in pain alleviation for many patients. CPSP patients can have a lower quality of life and experience difficulty even doing simple tasks. Now there is a shift in research to explore preventative measures and explore possible risk factors and this project reflects this shift. There is a need for better intervention plans for patients at risk. In the current study we examine the psychological and environmental effect on pain and overall well-being pre- and post-surgery in a mouse population. While there are studies of the effect of depression on pain in mice very few have studied chronic neurological pain along with overall well-being. We will be one of the first to include an enriched environment to our research. There are also few studies that take into account prior well-being and the effect it can have on pain responses post surgery. Our research will also study the effect of a reduced stress environment on well-being and pain response.

By identifying mechanisms of how environment can have an effect on mental health and how in turn that can affect well-being and pain perception we hope to be able to identify factors that put patients at increased risk for developing chronic post surgical pain. In doing so physicians and therapists can provide patients with improved behavioral interventions prior to surgery that will facilitate a better surgery outcome. The role of psychosocial factors in the development of CPSP is recognized but the mechanism of action has not been elucidated. Using an animal model to better understand the relationship of environment and psychosocial factors with chronic pain will allow
researchers to apply those concepts when interpreting patient data. This experiment will also set the pace for future research on the effect environment and psychosocial factors have on expression of genes associated with pain perception.

We aim to determine whether pre-surgical environment will predict overall well-being and pain sensitivity before and after a surgical procedure. We aim to explore the effect of depression on evoked pain behavior in mice and pain sensitivity. We hypothesize that mice exposed to chronic mild stress will exhibit abnormal social behaviors, will display more timid behaviors, and will have lower thresholds to pain than the control and enriched mice. We also hypothesize that differences in behaviors and pain threshold will be stronger post surgery. As enriched mice will be in a reduced stress setting we hypothesize that pain threshold may be higher for these mice and that more timid behaviors will be reduced.
EXPERIMENTAL METHODS

Thirty adult, male, C57BL6/J mice were used. Ten mice were exposed to each environment (chronic mild stress, control and enriched cage) with food and water available ad libitum (except where stressed mice had food or water removed as part of the stress paradigm). Lights were on from 07:00h to 19:00h (except where stressed mice had lights left on for 24h as part of the stress paradigm).

On the 3rd week mice were tested to obtain baseline behavioral and pain threshold scores. On the 5th week mice underwent spared nerve injury (SNI) surgery. Following surgery the mice remained exposed to their respective environments and behavioral experiments and pain threshold experiments were repeated.

Environment

Chronic Mild Stress: Mice were housed in standard opti-cages and exposed to one or two random stressors a day.

Control: Mice were housed in standard opti-cages in mobile racks.

Reduced Stress/Enriched: Mice were housed in large cages; 10 mice per cage. The cage was divided into two levels connected by a ladder or chute. The top level of the cage contained a maze which may be changed with 5 other designs to provide the mice with variety. The bottom level of the cage contains 3 running wheels and 3 water bottles in one compartment and a small housing unit and a food hopper in another compartment. Motivation for mice to move through the maze is the need to find food and water which
are placed in two separate compartments. Marlau cages are designed to encourage cognitive enrichment. One way doors separate these two compartments, encouraging mice to climb to the second floor, go through the maze, and down the chute in order to access food after drinking water.

**Chronic Mild Stress Procedure**

Over a 6 week period mice were exposed to one or two of the following mild stressors per day at random (Table 2).

**Table 2: Mild Stressors Used on Mice**

<table>
<thead>
<tr>
<th>Stressor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overnight Illumination: Mice were left in an illumination box for 12 hours with food and water.</td>
</tr>
<tr>
<td>Food Deprivation: Mice in opti cages were placed on racks without access to food hoppers overnight.</td>
</tr>
<tr>
<td>Water Deprivation: Mice in opti cages were placed on tabled and deprived from water access for 8 hours during the daytime.</td>
</tr>
<tr>
<td>Forced Swim: Mice were placed in large beakers of cool water (approx. 13°C) which they could not escape from for a 5 minute time period.</td>
</tr>
<tr>
<td>Moist bedding: Bedding in opti cages was moistened by emptying 400ml of water onto the bedding and mice were left for 8 hours.</td>
</tr>
<tr>
<td>Cage Reduction: cage dividers were placed in opti cages and bedding removed from one side of the cage. Mice were placed in the side of the cage without bedding for 8 hours.</td>
</tr>
<tr>
<td>Cage Shake: opti cages were shaken horizontally for 5 minutes with mice inside them.</td>
</tr>
</tbody>
</table>
Table 3. Schedule of Unpredictable Chronic Mild Stress model and Experiments along with a key.

<table>
<thead>
<tr>
<th>WEEK</th>
<th>TYPE OF TEST</th>
<th>TESTS PERFORMED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Scheduled CMS</td>
<td>CS; S; WD; FD; CT; M; O; SR</td>
</tr>
<tr>
<td>2</td>
<td>Scheduled CMS</td>
<td>CS; S; WD; FD; CT; M; O; SR</td>
</tr>
<tr>
<td>3</td>
<td>Scheduled CMS and Pain Tests; Behavioral</td>
<td>CS; S; WD; FD; CT; M; O; SR; Von Frey; Heat Place Preference; Hold</td>
</tr>
<tr>
<td>Day</td>
<td>Tests</td>
<td>Board</td>
</tr>
<tr>
<td>-----</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>4</td>
<td>Scheduled CMS and Pain Tests; Behavioral Tests</td>
<td>CS; S; WD; FD; CT; M; O; SR; Hot Plate; Social Interaction</td>
</tr>
<tr>
<td>5</td>
<td>SURGERY; CMS; Pain Tests</td>
<td>SNI on days 1 and 2; CS; S; WD; FD; CT; M; O; SR; Von Frey</td>
</tr>
<tr>
<td>6</td>
<td>CMS; Pain Tests; Behavioral Tests</td>
<td>CS; S; WD; FD; CT; M; O; SR; Von Frey; Dynamic Weight Bearing; Holeboard</td>
</tr>
<tr>
<td>7</td>
<td>CMS; Pain Tests; Behavioral Tests</td>
<td>CS; S; WD; FD; CT; M; O; SR; Von Frey; Social Interaction; DWB</td>
</tr>
</tbody>
</table>

### Stressors

<table>
<thead>
<tr>
<th>CS</th>
<th>Cage shaking (1 time/cage for 5 min) between 9 and 5pm (CS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>A cold swim (13 ± 1 C, 5 min/mouse) between 9 and 5pm (S)</td>
</tr>
<tr>
<td>SR</td>
<td>Space reduction with no bedding between 9am and 5pm - half of opticage divided length ways (to allow water and food access). (SR)</td>
</tr>
<tr>
<td>WD and FD</td>
<td>Water deprivation (WD) (8 hr) between 9am and 5pm followed by food deprivation (FD) 16hrs (5pm-9am) (WFD)</td>
</tr>
<tr>
<td>CT</td>
<td>Cage tilting 45 degrees (8 hr/cage) between 9am and 5pm (CT)</td>
</tr>
<tr>
<td>M</td>
<td>Moist bedding (8 hr/cage) between 9am and 5pm (M)</td>
</tr>
<tr>
<td>O</td>
<td>Overnight illumination (12 h) (O)</td>
</tr>
</tbody>
</table>
Behavioral Tests

Holeboard Experiment:

The holeboard was a square arena with 9 holes in the floor (arranged 3 x 3). Horizontal, vertical and holepoking activity were detected using infra-red beams. Lights were dim (approximately 30 lux) in order to produce a reduced stress environment for the mice. Mice were placed in holeboard boxes and tested for 15 minutes. The number and duration of head dips into holes was measured along with time spent in the periphery and the center of the box.

Social Interaction Experiment:

The arena was a rectangular box divided into 3 equal chambers connected by a small aperture. A test mouse was placed in the central chamber and allowed to explore the whole arena for 5 minutes with all doors open. After 5 minutes the experiment was paused and a novel mouse was placed in a cylindrical container and introduced to either the right or left chamber of the arena and an empty container was placed in the opposing chamber. The test mouse was then placed back into the central chamber and allowed to explore the arena once again with all doors opened for 10 minutes. The amount of time the test mouse spent in close proximity to the novel mouse and the empty container was measured using Ethovision (to reduce subjectivity of observation) and researchers were blinded to the test groups of the mice. This side the novel mouse was introduced to was
varied each time (right, left, right, left etc). This was done to reduce favorability of the mouse to go to one side due to mouse scent etc.

**Pain Tests**

*Thermal Place Preference*

Two hot plates were placed side by side. The trial started with one plate at 30°C and the other at 49°C. Both plates changed temperature according to a heat ramp such that plate 1 increased to 49°C and plate 2 decreased to 30°C. This cycle was repeated so that over a 15 min period both plates returned to their original temperature. Over the course of the test the mouse was allowed to travel from one plate to another and back again and time spent on each plate was captured using Ethovision.

*Hot Plate*

Mice were exposed to a hot plate and the time taken for the mouse to begin licking its feet was measured. A maximum cut off of 20 seconds was used to prevent any mice from suffering permanent tissue damage.

*Dynamic Weight Bearing*

The dynamic weight bearing device measured the weight distribution of the mouse across each paw using Pressure transducers were located on the floor of the device. Mice were allowed to move freely on the floor of the device for 3 minutes.
**Von Frey Test**

Mice were placed into plastic chambers and allowed to habituate for 30 minutes in order to reduce stress interference with pain threshold measurements. Following habituation filaments were applied to the ventral side of the rear foot (left hind paw for injured animals) in increasing filament size (0.02-4g). Each filament was applied 10 times before moving to a higher filament. A positive response was taken to be paw withdrawal 5 or more times out of the 10 applications of the filament. This force value was taken to be the pain threshold. Heavier filaments were applied to confirm the threshold value. Testing was performed both before and after nerve injury.

**Spared Nerve Injury**

A spared nerve injury was performed on all mice (n=30) on week 5 of the experiment. Mice were placed under anesthesia (Isoflurane 3.5% induction, 1.5% maintenance) and an incision on the lateral side of the left thigh was made. The biceps femoris muscle was separated in order to expose the sciatic nerve and its three terminal branches, the sural, common peroneal, and tibial nerves (Figure 4). The peroneal and tibial nerves were tightly ligated using 6-0 silk and axotomy was performed distal to this, removing approximately 2-4mm of the distal nerve stump. Care was taken not to injure or stretch the intact sural nerve. Mice were placed back into a clean home cage (opti cage or enrichment cage) and following recovery from anesthesia, placed back in the regular housing room. The animals were left to recover for 7 days before Von Frey testing was performed weekly for 3 weeks.
Figure 4. Schematic diagram of the sciatic nerve and its three branches in the paw of a rodent. A schematic diagram of the sciatic nerve and its three branches in the paw of rodents (sural, tibial, and common peroneal). Both the common peroneal and tibial branches were severed, leaving the sural branch intact. (Figure Amended from Spared Nerve Injury: an animal model of persistent peripheral neuropathic pain[48]).
RESULTS

PRE-SNI DATA

Hot Plate

It was found that chronic mild stress mice had a shorter average response time to hot plate at 52°C than control or enriched mice (Figure 5). Control mice had an average of 9.2s with a standard error mean of 0.6s, enriched mice had a slightly lower average of 8.7s with a standard error mean of 0.8s; however CMS mice had an average response time of 7.6s with a standard error mean of 0.5s. A significant difference was seen between control and CMS mice withdrawal latency with p<0.05 using a one way ANOVA post hoc.
Figure 5. Hot Plate Response times of mice at 52°C. Bar chart representing the average response times to hot plate at 52°C. Blue represents control mice, pink represents chronic mild stress, and orange represents enriched mice.

**Dynamic Heat Place Preference**

No significant difference in average time spent on the hot plates at a given series of temperatures was found between the three groups of mice (enriched, control, and chronic mild stress). It was found that mice avoided the plate at higher temperatures and spent more time on the plate during lower temperatures. Dynamic Heat Place Preference in used to measure threshold to temperature (Figure 6).
Figure 6. Average amount of time spent on plate at a given temperature of enriched, control, and chronic mild stress mice. Average time spent on the hot plate at a series of given temperatures, enriched mice are shown in green, control mice are shown in blue, and CMS mice are shown in red.

**Holeboard Experiment**

In the holeboard experiment we used infrared beams to study distance traveled and amount of time spent in the periphery versus the center of the board. It was found that CMS mice traveled less while in the center of the board and more while on the periphery than enriched or control mice. CMS mice had an average ratio of distance in center:distance in center of 13.92 whereas control and enriched had ratios of 18.86 and 18.57 respectively (Figure 7). There was a significant difference of the distance traveled in the center: distance traveled in the periphery between CMS and enriched mice with a p<0.05 one way ANOVA. It was also found that CMS mice spent less time in the center.
and more in the periphery compared to control and enriched mice with a significant
difference found between CMS and control mice with a p<0.05 one way ANOVA. CMS
mice were found to have a ratio of 8.97 of time spent in center: time spent in periphery,
whereas control mice had a ratio of 12.99 and enriched mice had a ratio of 12.74. CMS
mice were found to spend a lower percentage of their total time in the center of the arena
(8.15%) than enriched (10.91%) or control mice (11.22%).

Figure 7. A bar chart showing ratio of distance travelled in center:distance traveled
in periphery of holeboard experiment. The ratio of distance traveled while in the center
of the area: the distance traveled while in the periphery of the arena of CMS mice
(center), control mice (left), and enriched mice (right).
Figure 8. A bar chart showing a ratio of time spent in center: time spent in periphery of mice in the holeboard experiment. The ratio of time spent in the center of the arena: the time spent in the periphery of the arena of enriched mice (right), CMS mice (center), and control mice (left) during the holeboard experiment.
Figure 9. Percentage of total time was spent in the center of the holeboard. The percentage of time in the arena that was spent in the center. Stressed mice are shown in the center, enriched on the right, and control on the left.

Von Frey

Baseline Von Frey scores were collected before SNI surgery (Figure 10). It was found that threshold values were between 1.4g and 2g.
Figure 10. Baseline Von Frey Values Pre SNI, The average number of responses at given Von Frey fiber weights. Responses given 5 or more times out of 10 applications are considered to be positive and that weight is taken as threshold value. CMS mice are shown in red, control mice in blue and enriched mice in green.

Social Interaction

It was found that mice tended to avoid the novel mouse in favor for the empty chamber that had recently had the previous rounds’ novel mouse. It also appeared that CMS mice had a greater reduction in mouse interaction post SNI surgery than control and enriched mice.
Figure 11. Habituation times in empty and novel mouse chambers of mice pre- and post-SNI surgery. Habituation times in each chamber of CMS mice (red), control mice (blue) and enriched mice (green) are shown in Figure 10. Measurements of time were taken before and after SNI surgery. During these measurements no novel mouse was present in the arena.

Figure 12. Interaction times in empty and novel mouse chambers of mice pre- and post-SNI surgery. Social Interaction times in each chamber of CMS mice (red), control mice (blue) and enriched mice (green) are shown in Figure 10. Measurements of time
were taken before and after SNI surgery. During this time measurement the novel mouse was present in the arena.

**POST- SNI DATA**

Many procedures were not repeated post SNI in order to reduce confounding variables with Von Frey results.

*Dynamic Weight Bearing*

During post SNI dynamic weight bearing revealed that mice from all three groups (CMS, enriched, and control) placed more weight on their hind right paw than their hind left paw (Figure 12). Hind left paw was injured in surgery. It was also found that as time progressed less weight was put on the left hind paw and more on the right hind paw (Figure 13). Differenced between rear paw weight distribution (L-R) were -5.71 for CMS mice, -7.01 for control mice, and -6.79 for enriched mice at day 8 post SNI. However, after 16 days this difference decreased in control mice to -6.71 and increased in CMS and enriched mice to -6.79 and -9.69 respectively.
Figure 13. Weight bearing on left and right hind paws on days 8 and 16 post SNI. The average amount of weight distributed on the left and right hind paws on days 8 and 16 post SNI surgeries. Control mice are on the left, CMS mice in the center, and enriched mice on the right.
Figure 14 Weight distribution ratios between left and right hind paws on days 8 and 12 post SNI. Ratio of left:right hind paw weight distribution in control mice (left), CMS mice (center) and enriched mice (right) on days 8 and 16 post SNI surgery.

*Post SNI Von Frey*

Post spared nerve injury (SNI) it was found that the number of average Von Frey responses over the series of applied weight remained the same. There was no significant difference in the average number or responses for any given weight (data not shown).

**DISCUSSION**

In this study we investigate the role that environment and psychosocial factors can play on pain perception and overall well being. It seems that depression and chronic stress can have an effect on thermal pain threshold and induce more timid and anxious behaviors while having no effect on others.

**Hot Plate**

Chronic Mild Stress (CMS) mice had a significantly reduce withdrawal time than control mice indicating a hypersensitivity to thermal stimuli and lower thermal pain threshold. CMS mice also had a lower average withdrawal time than enriched mice, indicating that environment may have played a role in pain threshold and sensitivity. One of our aims is to use animal models to further understand effect of environment on pain threshold and sensitivity. These results were as we expected; it has been found in other studies performed by Crettaz et al that acute stress can lead to and increased sensitivity to thermal pain and that patients who experienced chronic pain also had higher thermal pain
sensitivity. This result has been seen in other animal studies as well; in a study by Imbe et al it was found that stress enhanced thermal hyperalgesia and the phosphorylation of CREB. CREB is often phosphorylated as a result of cAMP associated with mechanical hyperalgesia. Therefore it can be deduced that CMS mice are experiencing hyperalgesia due to stress and depression caused by the chronic mild stress model.

**Dynamic Heat Place Preference**

Mice from all groups avoided higher temperatures as seen with Figure 2. Heat Plate Preference is seen to measure heat threshold as mice often move before the heat stimuli becomes noxious. It can be inferred from the graph that mice from all three groups have similar heat stimuli sensory thresholds and experience hot and cold stimuli normally. This may imply that while chronic stress and depression may have an effect on thermal pain threshold, it may not have an effect on heat threshold.

**Holeboard Experiment**

Distance moved and time spent moving are among a number of things evaluated in an open field test. Many factors such as exploratory drive, anxiety, freezing, sickness, and other fear-related behaviors can have an effect on movement. Center versus periphery time is used to gauge the emotional state of the animal such as anxiety. It is thought that rodents who spend more time exploring periphery and near edges tend to be more anxious and timid while those who spend more time exploring in the center of an arena are more driven to explore and less timid. We found that CMS mice spent less time exploring the center of an arena than control and enriched mice; leading us to believe that
CMS mice are more timid and anxious. This anxiety and timid behavior may be a result of the chronic mild stress procedure and it may be inferred that the procedure succeeded in producing abnormal emotional states and behavior in mice that may be mimicked in humans.

**Social Interaction**

During interpretation of results it seemed that mice, when first introduced to the center chamber for habituation that mice from all groups (CMS, Control, and Enriched) tended to avoid the chamber that the novel mouse would be introduced to. It was hypothesized that control mice would spend more time in the chamber containing the novel mouse than in the empty chamber; this discrepancy between expected results and actual data lead to further investigation of procedure. As novel mice were introduced to the chamber at alternating sides each time it was determined that mice were attracted to scent left behind by a most recent novel mouse used; cages used for novel mice were cylindrical it was difficult to completely clean them of all scent. Another problem that arose when obtaining data was that Ethovision would lose track of the experimental mouse due to the mouse climbing out of view and onto the novel mouse cage or crawling behind the novel mouse cage. Therefore much of the data was corrected for and a number of runs could not be used. In the second round of mice experiments these problems will be addressed. However, a trend was seen that CMS mice tended to avoid novel mouse interaction to a greater extent than control or enriched mice. This timid behavior could be indicative of anxiety or stress. Abnormal social behaviors and avoiding other mice may also be a characteristic or depression induced by CMS model.
Von Frey
Surprisingly, our post SNI Von Frey results were not as expected. In accordance with literature on similar experiments performed we expected post SNI Von Frey average responses to be higher on fibers of lower caliber, indicating signs of hyperalgesia. Higher number of responses on lower weights would have indicated a lower threshold to pain as is expected with neurological chronic pain. It is believed that surgery was successful in producing chronic pain as there were visual cues such as twisting of the foot upon walking and less weight placed on the injured foot that indicated so. Mice from all three groups (CMS, Control, and Enriched) all produced average responses similar to those before surgery leading us to believe that they all developed hypoalgesia. While the reason for this occurrence remains a mystery the study will be repeated again to obtain more data.

Dynamic Weight Bearing
Looking at dynamic weight bearing it is apparent that SNI surgery was successful as CMS, control, and enriched mice all reduced the amount of weight they placed on their left hind paw and increased weight bearing on the hind right paw. This indicated that the left hind paw was injured and placing weight on it might have been painful for mice in all groups. Results also indicate that pain experienced by mice was persistent because decreased weight bearing on the injured paw and increased weight bearing on non-injured paw were seen at 8 and 16 days after SNI surgery. For both enriched and CMS mice the difference in weight bearing between both hind paws got even greater after the 8 day measurement and weight these mice distributed on their left hind paw decreased. This
indicates that pain may have worsened or pain coping mechanism may no longer have worked as well. As CMS model was continued post surgery the continued stress may also have contributed to this increase difference in weight bearing.

**Future Directions**

Our current study aim is to determine whether pre-surgical environment (control, CMS, or enriched) would have an effect on overall well-being and pain sensitivity pre and post surgery in adult male mice. Since this preliminary data suggested a correlation between pre-surgical environment and pain sensitivity we will continue these studies to test for significance. Additionally, we will also be testing for anhedonia via sucrose drinking test to confirm CMS induced depression in experimental mice. Future studies will hope to identify whether the environment will alter gene expression of genes associated with pain perception leading to changed risk profiles associated with chronic post surgical pain. An example of this is the GCH1 gene which codes for GCH1, an enzyme required for the synthesis of tetrahydrobiopterin (BH4). Elevated levels of GCH1 and BH4 have been associated with chronic pain and GCH1 gene expression has found to be altered by a number of inflammatory mediators. The hypothesis that chronic stress can induce altered gene expression has been studied; it was found that chronic stress can induce IL-1β levels in the spine to rise which may in turn induce elevated levels of GCH1. The hypothesis that environment can have an effect on gene expression will be headed by Dr. Christine Sieberg and Dr. Michael Costigan at Boston Children’s Hospital Pain Core & Neurodevelopmental Behavior Core. Effects will be observed using gene knockout and over expression models of the GCH1.
We hope to also replicate this experiment in young mice (3-5 weeks old) in order to better assess factors contributing to pediatric chronic post surgical pain.

The use of a mice model to elucidate the mechanisms in play is critical however a better understanding of patient related data must follow to substantiate these findings. As such, blood samples collected at Boston Children’s Hospital from patients undergoing spinal fusion surgeries will allow for genetic analysis of patients. We can take concepts of environmental effect on gene expression and investigate the same effects in humans from blood samples. Understanding the mechanism of action in the environment-pain relationship will allow for better intervention techniques and screening for at-risk patients.

Future approaches in prevention might be geared towards the prevention of microglial cell activation. Microglia cells are responsible for the induced hypersensitivity (via transcriptional changes) in neurons of the dorsal horn. Minocycline has been used as an antiinflammatory agent in order to stop microglial activation. Use of this drug before anticipated nerve injury can prevent the development of hypersensitivity\textsuperscript{2}. During allodynia resulting from nerve injury ATP is released and acts on purinergic noicceptive receptors in the dorsal horn and spinal cord. Blocking these receptors may diminish or prevent pain associated with nerve injury\textsuperscript{54}. Other future targets in prevention may also be blocking sodium channels; in animal models it was found that selective sodium channel blockers may reduce chronic pain in rats\textsuperscript{55}. 
While Von Frey results leave room for more investigation it is apparent that CMS-model-induced-depression had an effect on thermal threshold and dynamic weight bearing while also producing anxious and more timid behaviors in the mice exposed to this paradigm. With a second round of mice we hope to uncover a deeper relationship between environment and chronic pain and further understand how this mechanism may work.
REFERENCES


CURRICULUM VITAE

ARCHANA REDDY

Year of birth: 1991
26078 N Kings Mill Lane
Kingwood, TX 77339
areddy2@bu.edu

EDUCATION:

University of South Florida
Tampa, FL
Bachelor of Science in Biology with Honors, May 2012
Dean’s List: Spring 2011 – May 2012, GPA: 3.66
Scholarships: Green and Gold Scholarship

Boston University School of Medicine
Boston, MA
Candidate, Masters in Medical Sciences, May 2014
GPA: 3.66

RESEARCH: Boston Children’s Hospital Department of Anesthesiology (January 2013-present)

Research in the Anesthesiology department at Children’s Hospital of Boston as a research assistant. Her project focuses on pain processing in children who undergo spinal fusion surgery as treatment for idiopathic scoliosis. She has been trained in Quantitative Sensory testing under which pain threshold will be measured in children. She has obtained experience in collecting and organizing patient information, performing chart reviews, working with SurgiNet, working with patients, conducting data collection, statistically analyzing data collected, and working within a team. I have also been trained to work with rodents.

EXPERIENCE:

Boston University Tutor (Sept 2013-present)

Tutor for the Boston University School of Medicine. She tutors Dental students and GMS Masters students in Physiology and Biochemistry for up to 10 hours a week.
Resident Assistant Fall 2010-Spring 2012  
University of South Florida Residence Hall  
- Participated in organizing resident programs, supervising a floor of 34 students, developing effective communication skills, building a resident life community. Obtained organizational and hands on people skills.  
- Customer service to current and prospective residents, working to sell housing leases to prospective students.

Hospital Volunteer Spring 2011-Spring 2012  
James A. Haley Veteran’s Hospital, Tampa FL  
- Volunteered in Emergency Room. Obtained hands on people skills and patient interaction. Worked with many physicians and nurses in the ER.

Family Clinic Volunteer Summer 2009-Spring 2012  
Flamingo Gardens Clinic, Nassau, The Bahamas.  
- Volunteered at a Family clinic with a gynecologist. Obtained experience in patient interactions, gained insight to doctor-patient relationships and developed hands on people skills. Involved with team planned family planning clinics and presented information to patients at family planning clinics.

Volunteer Rotaract Fall 2009-Spring 2012  
- Volunteered with Rotaract, a community service organization. Spent weekends volunteering with IronKids, Shriner’s Children’s Hospital, Beach Clean ups, Fundraising events, and the Children’s Cancer Society.

Volunteer School Supplies for Afghan Children Fall 2011-Spring 2012  
Held fundraising events in which we collected money and school supplies that were packed and shipped to humanitarian troops in Afghanistan. The school supplies were distributed to boys and girls in rural parts of Afghanistan.

Volunteer American Veterans with Brain Injuries Fall 2011-Spring 2012  
Worked to fund raise for veterans who return from Afghanistan with Traumatic Brain Injuries. Has organized many fundraising events and educational events in order to collect money for the charity. Money is used
to provide these veterans with a brain training program along with dog tags and medical cards that help others identify their medical condition. Has learned organizational skills and have gained knowledge on brain injuries.

**EXTRACURRICULAR:** University of South Florida Swim Team Fall 2011-Spring 2012

USF Swim Team, a small non-sponsored team. Practices 5 days a week for an hour. Competed in meets at University of Florida, Florida State University, and other various independent meets.