The Effects of Environmental Cross-Over on Inflammation-Induced Nociception

by

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ABSTRACT

There are many studies in which environmental enrichment (EE) has focused on modifying animal behavior leading to improved cognitive functions, such as learning and memory. There are also increasing numbers on how EE positively affects a subject's pain tolerance by increasing thresholds. In order to better understand how an environment can affect nociceptive behaviors, this study looked at the effects of cross-over from one environment to another after induced inflammation. Male Sprague-Dawley rats were initially randomly assigned three to a cage; four of the cages were standard, only containing bedding and food/water, while the other four cages were supplemented with toys (enriched). Baseline behavioral measurements, paw thickness, paw withdrawal latency (PWL) and paw withdrawal threshold (PWT) were collected. After five weeks, CFA was injected into the left hind paw of all 24 rats and the cross-over was conducted. Two of the initial standard cages stayed standard, while the remaining two standard cages changed to enriched. Two of the initial enriched cages changed to standard, while the remaining two enriched cages stayed enriched. The behavioral measurements were then conducted post CFA at 2 hours, 7 days, 14 days, 21 days, 28 days, and 35 days. A repeated measures ANOVA was conducted on paw thickness for all four treatment groups (standard-standard, standard-enriched, enriched-standard, enriched-enriched) at baseline, 2 hours, 7 days, 14 days, 21 days, 28 days, and 35 days post-inflammation. $F_{10.125,44}$=1.403 with no significant difference among home cage treatment groups (p-value = 0.180). There was, however, a significant difference across testing times (2h, 7d, 14d, etc.; p-value < 0.001) and $F_{3.485,44}$=1.403. The repeated measures ANOVA conducted on PWT revealed $F_{3.801,44}$= 2.520 and no significant difference among the four housing groups

(p-value = 0.311). There was a slight significant difference across behavioral testing times (p-value = 0.046), and $F_{11.402,44}$=1.169. The repeated measures ANOVA conducted on PWL showed no significant difference among home cage housing groups (p-value = 0.446) and $F_{11.244,44}$=1.004.There was a significant difference across behavioral testing times (p-value < 0.001) and $F_{3.748,44}$=22.405.

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Chapter 1

I. Introduction

A. Pain: The Sensory Experience

Historically, pain was treated as a subcategory of somatic sensation (Kandel et al., 2000), but studies conducted on this modality have concluded that pain must be viewed as a distinct sense. Pain is a highly individualized experience and is modifiable (Melzack, 1961). Pain's variable nature is due to a number of reasons, including the capacity of an individual to deal with a stressful situation, both the internal (endogenous hormones) and external (exogenous medications) algesic and analgesic chemicals, and the individual's personal characteristics such as sex, age, and personality (Ramirez-Maestre et al., 2003). The most fitting definition for pain is that it is a complex phenomenon of unpleasant sensory, emotional, and cognitive experiences that are brought about by real, potential, or perceived tissue damage (Terman and Bonica, 2003). Physiological pain is known to be a direct result of tissue damage and serves as an adaptation, or alert system, to make organisms aware of noxious stimuli by triggering negative, affective responses. It is important to remember that pain is a heavily modulated sensation and is integrated with many other systems of the brain, rather than a separate sense functioning in isolation (Shepherd, 1994).

1. Inflammation and Pain

Tissue damage causes inflammation, a nonspecific immune response. There are four cardinal signs and symptoms of localized inflammation: pain, swelling, redness, and heat. Inflammatory pain is caused by a variety of chemical factors released by damaged cells, called the "Inflammatory Soup" (e.g., prostaglandins, Substance P, hydrogen ions, bradykinin), that leads to neuroplastic changes. These

changes consist of an increase in sensitivity by afferent neurons in the peripheral nervous system (PNS), and an increase in sensitivity of spinal cord dorsal horn neurons as well as other neurons in the central nervous system (CNS; Ji, 2004).

Upon induction of the inflammatory response, the immune system responds with both direct and indirect defenses in order to combat the changes that occur during inflammation. Direct defense consists of the phagocytosis of damaged cells, while the indirect defense consists of two branches; innate immunity and adaptive/acquired immunity. The major cell type that plays a precipitating role in all three defense systems is the mast cell. These cells serve to give the body the ability to resist toxins/organisms that are capable of causing damage to tissues and organs. Mast cells are spread throughout connective tissues of the body, especially beneath the surface of the skin, near blood vessels and near lymphatic vessels. These cells synthesize and release heparin, a weak anticoagulant, and histamine, a chemical mediator of inflammation which increases vascular permeability (Eroschenko, 2005). With this increased permeability, excess fluid leaks out of circulation into the interstitium and induces edema. Along with increased vasodilation, edema accounts for the swelling, redness, and warmth associated with localized inflammation. Serotonin, bradykinin, and a variety of prostanoids are also released by mast cells at the site of local inflammation (Fearon and Locksley, 1996).

Prostanoids are a family of lipid based molecules that are derived from the action of cyclooxygenases or prostaglandin synthases on eicosanoids. Prostanoids can be divided into three groups, prostaglandins**,** prostacyclins, and thromboxanes (Nicolaou, 2004). Prostanoids are active in processes such as blood pressure regulation, hemostasis, and production of the inflammatory response. During inflammation, prostanoids are synthesized in greater quantities than what is normally

present during basal conditions within the body. When a tissue is exposed to a noxious stimulus, such as a sprain or strain, arachidonic acid is released from cell membrane phospholipids by activation of the enzyme phospholipase A2 (Smith, 1992), due to an increased calcium ion influx. Arachidonic acid is converted to prostaglandin H_2 (PGH_2) by prostaglandin G/H synthase 1 or 2 and PGH_2 is then converted into the active, paracrine prostanoid chemicals. There is growing evidence that show that prostaglandin G/H synthase 1 or 2 -derived prostaglandins are critical in many cellular processes including cell proliferation, differentiation, and apoptosis, which are all inflammation-associated responses. In addition, prostaglandins are associated with the pathogenesis of cancer, cardiovascular disorders, and inflammation (Zhang et al., 2010). Prostaglandin E_2 (PGE_2), an active prostanoid, is critical to the pain associated with inflammation by increasing the responsiveness of peripheral nociceptors in inflamed tissues, and is formed via the conversion of PGH_2 by PGE synthase.

Once PGE_2 is formed, it is transported through the membrane of the cell where it is produced by the ATP-dependent multidrug resistance protein-4 in order to act on the cell it was secreted from (autocrine action) or near (paracrine action) its site of secretion (Park et. al, 2006). PGE_2 acts via four subtypes of metabotropic receptors named E-prostanoid receptors, or EP_1-EP_4. The binding to EP_1 receptors on the primary afferent neuron plays a major role in hyperalgesia, a classic symptom of inflammation that will be subsequently discussed, by acting on peripheral sensory neurons innervating structures at the site of inflammation (Moriyama et. al, 2005; Dubois et. al, 1998). Studies have shown that EP_2 receptors located in the dorsal horn of the spinal cord, and EP_4 receptors located on the primary afferent neuron, are significantly upregulated in induced paw edema in rats (Yuhki et. al, 2004), showing

the importance of PGE_2 in mediating inflammation. Activation by PGE_2 of EP_1 –EP_4 receptors on pain sensing neurons results in a lowered action potential threshold, allowing for a painful response to be elicited under innocuous situations or exaggerated. Behaviorally, this effect is observed as hyperalgesia and allodynia.

Sensitization describes a change in neuronal function following repeated stimulation. When a pain signal activates an afferent neuron, there is an increase in neuronal excitability and a lowering of pain thresholds, allowing for an increased response to stimuli (Woolf, 1983; Woolf and Thompson, 1991; Dubner and Ruda, 1992; Coderre et al., 1993), resulting in hyperalgesic and allodynic responses. These altered pain-related responses develop in animal and human subjects following a cutaneous injury (Cervero and Laird, 1996). Tissue damage often leads to an increase in the response to subsequent noxious stimuli, a phenomenon called hypersensitivity or hyperalgesia. Allodynia, also a result of sensitization, is a painful response to a stimulus that would not normally produce pain, or an innocuous stimulus. Hyperexcitability of neurons related to the sensation of pain is produced by local inflammation.

Acute, localized inflammation normally has a beginning and an end. The temporal aspects of the inflammatory response are mediated by the synthesis and release of chemicals that are pro-inflammatory and anti-inflammatory, respectively. Alterations to these temporal processes can lead to long-term, maladaptive inflammation. Sub-chronic inflammation is defined as inflammation lasting longer than acute inflammation, but shorter than chronic inflammation, usually around 6-12 weeks. Chronic inflammation occurs when an inflammatory response is not terminated, so there is a continued production of pro-inflammatory, chemical mediators. This type of inflammation lasts longer than 12 weeks. Symptoms of

chronic inflammation include systemic effects such as body aches, sinus congestion, shortness of breath, swelling, stiffness, and weight gain (Reasoner, 2010). Normally the biochemical reactions of inflammation cease when homeostasis is restored, but in the case of pathological chronic inflammation, the communication is disrupted and the reactions do not "turn off" (Pick, 2011). Eventually, through temporal release, the inflammatory response does cease in chronic inflammation, going into remission. However, relapse is common.

2. Experimental Models of Inflammation

In order to gain a better understanding of the signs and symptoms of inflammation, experimental animal models using a single injection of a pro-inflammatory chemical into the hind paw or joint have been developed (Ji, 2004). The development of reproducible animal models of human ailments has been crucial to gaining a better understanding of underlying pathophysiologic factors and has contributed to the development of more effective treatments. One of the most commonly used agents in preclinical investigations has been Complete Freund's Adjuvant (CFA; Stein, 1985). CFA is an injectable mixture of heat-killed bacteria suspended in sterile mineral oils that induces a sub-chronic inflammatory response (Freund, 1947). Another experimental model used to induce acute inflammation is an injection of carrageenan, a method originally developed as an effective way to standardize testing of anti-inflammatory drugs (Winters, 1962). Carrageenan and CFA are well-established experimental models of inflammation with a long history of effective use in preclinical studies, producing the same signs and symptoms associated with localized inflammation as mentioned previously.

Actual and experimentally-induced inflammation results from tissue damage and elicits the sensation of pain. The ascending pain pathway is a three neuron

journey to the cerebral cortex with collateral branches transmitting noxious input to accessory areas of the brain as well as activating endogenous analgesia.

B. Anterolateral System (ALS)

The circuit anatomy of nociception in the human body consists of neurons and pathways leading to the cerebral cortex. The ALS (Fig. 1) is a group of sensory pathways ascending the spinal cord in the anterolateral portion of the white matter that conveys information about temperature and noxious stimuli to the brain. The ALS contains three pathways, including the spinothalamic (neospinothalamic) pathway, the spinoreticular (paleospinothalamic) pathway and the spinomesencephalic pathway. The cutaneous nociceptive pathways begin at primary afferent nociceptors, neurons with cell bodies located in the dorsal root ganglion. The central axonal process of this neuron synapses onto the second order neuron in the dorsal horn of the spinal cord, which sends its axons rostrally as the spinothalamic tract. The neurons of the spinothalamic tract eventually synapse onto a third order neuron located in the thalamus. The third order neuron terminates on neurons of the primary somatosensory cortex in the parietal lobe of the cerebral cortex. Together these pathways allow the site of noxious stimulation to be localized, to be consciously perceived, and allow for the activation of the descending inhibitory pathway.

1. Nociceptor

The first step in the perception of noxious stimuli occurs at the level of the sensory neuron. The behavioral response to a noxious stimulus is a combination of PNS-related nociception and CNS-related pain perception. Nociception is elicited by noxious thermal stimulation, pressures and mechanical stimuli, or chemical stimulation. Nociceptors are a class of peripheral sensory neurons that exhibit high activation thresholds and preferentially respond to noxious stimuli (Basbaum, 1984).

It should be mentioned that "nociceptor" is not only a term for describing the sensory receptor located in the cell membrane of the afferent neuron, but is also the entire afferent neuron that carries the initial action potential as well.

a. Sensory Signal Transduction

The first process in nociception is the transduction of a noxious stimulus into an action potential. Neurons are excitable cells that have a resting membrane potential (RMP) between -60mV and -90mV established and maintained by ionic concentration gradients across the neuronal plasma membrane. Two important ions associated with membrane potentials are sodium (Na^+) and potassium (K^+). At the RMP Na^+ tends to leak into and K^+ tends to leak out of the neuron, while cells have protein pumps that compensate for the passive leakage by pumping K^+ into and Na^+ out of the neuron. The Na^+/K^+ ATPase pump, a primary active transport protein, uses the hydrolysis of adenosine triphosphate (ATP) to catalyze the movement of three Na^+ ions out of the cell for every two K^+ ions moving inward. The pump does not directly participate in the generation of action potentials, but serves to maintain the concentration gradients for Na^+ and K^+ across the cell membrane. In order for an action potential to be initiated in the peripheral axonal terminal of the nociceptor, the neuron must depolarize to a threshold potential (Hodgkin and Huxley, 1952). Depolarizing currents are produced by the movement of ions through a variety of plasma membrane-associated protein channels.

Transient receptor potential (TRP) channels are ion channels involved in the production of action potentials in nociceptive neurons. TRP channels are located within the plasma membrane of afferent neuron terminals and are non-selective cation channels, which when open permit the influx of Na^+ and calcium (Ca^{+2}). The TRPs resemble voltage-gated channels with the central pore closed at the RMP. Although

TRP channels may be weakly voltage-dependent, they lack the voltage sensor of voltage-gated Na^+ or K^+ channels. Instead, TRP channels act as sensors of osmotic pressure, temperature, and extracellular chemical changes. When homeostasis is disrupted, due to a noxious stimulus, TRP channel opening leads to a Na^+ and Ca^{+2} influx into the afferent neuron terminal, producing a depolarizing current. TRP channels are able to produce the initial depolarizing current because they are located on nociceptors themselves.

Ligand-gated ion channels open in response to the binding of an extracellular chemical stimulus and are referred to ionotropic receptors. These channels are formed by the non-covalent binding of several subunits facing one another to form an aqueous pore region. This pore can be highly selective for either Na^+, K^+, Ca^{2+}, or chloride (Cl^-). The depolarization produced from the TRP channels, mentioned abover, initiates the opening of voltage-gated Na^+ and Ca^{+2} ion channels, causing a further depolarization due to the additional influx of Na^+ and Ca^{+2}. If the depolarization is sufficient to reach the threshold voltage, an action potential is initiated.

Action potentials that carry nociceptive pain information are initiated in nociceptive neurons by noxious temperature, acidic chemicals such as hydrochloride (HCl), or noxious mechanical stimuli. At high temperatures, greater than 43º C, the rate of ionic diffusion increases, allowing for an increased influx of Na^+ via temperature-sensitive TRP channels. This channel opening leads to the depolarization and the production of an action potential in the nociceptor. When HCl is introduced to the interstitial fluid, there is an influx of H^+ through voltage-gated ion channels into the neuron. This positive influx results in nociceptor depolarization. Lastly, intense mechanical stimulation, such as hitting your thumb with a hammer, activates

mechanically-gated Na^+ channels resulting in the production of a depolarizing potential with sufficient magnitude to initiate an action potential in the nociceptive neuron.

b. Nociceptor- Primary Afferent Neuron

Action potential signals, no matter the sub-modalities of noxious stimuli, are carried into the CNS by primary afferent nociceptor neurons known as A-delta and C fiber nociceptors. C- fibers, or C-mechano-heat receptors, are polymodal neurons that are sensitive to noxious thermal, mechanical, and chemical stimuli (Table 1). The axons of these neurons are unmyelinated, giving them a relatively slow action potential conducting time (0.5 – 2 m/sec). This produces a "burning" sensation when activated (Shepherd, 1994). C-fiber central axon terminals are widely distributed (Dubin, 2010), but mostly terminate in the superficial layers of the spinal cord dorsal horn, such as the substantia gelatinosa.

A-delta fibers are thinly myelinated afferent nociceptors that can carry action potentials 5 to 30 meters per second, are generally responsible for noxious mechano-sensation and heat, and are responsible for the fast on-set of pain sensation (Table 1). A-delta fibers also synapse onto second order neurons within the substantia gelatinosa of the dorsal horn.

In 1965, Melzack and Wall introduced the Gate Control Theory of Pain (Melzack and Wall, 1965). This theory explains that nociceptive impulses are transmitted from peripheral nociceptor nerve endings within the skin to the substantia gelatinosa of the spinal cord dorsal horn through myelinated A-delta fibers and unmyelinated C fibers. The central axonal terminations and interneurons within this layer of the dorsal horn act as a "gate" which regulates the transmission of nociceptive impulses to the brain (Huether, 2004; Fig. 2). If an action potential is transmitted

through C fibers or A-delta fibers, the gate is “open,” allowing the pain information to be transmitted to the brain. If an action potential is also simultaneously conveyed by large, A-beta fibers, which carry information about touch, the gate is “closed,” blocking nociception and no signal is transmitted to the brain (Freudenrich, 2007).

There are two excitatory neurotransmitters, glutamate and substance P, that mediate the chemical neurotransmission between the primary afferent neurons and the second order neuron within the spinal cord. Glutamate is the primary excitatory neurotransmitter in the CNS (Meldrum, 2000). It is an amino acid-based neurotransmitter synthesized from glutamine by glutamine synthetase. Glutamate is released at the central axonal terminals of each nociceptor and it binds to receptors located on the second order neurons. There is approximately 100 mM of glutamate within each pre-synaptic neurotransmitter vesicle and the release of a single vesicle produces a small, depolarizing excitatory postsynaptic potential (EPSP) in the postsynaptic neuron. The simultaneous release of multiple synaptic vesicles and activation of multiple postsynaptic glutamate receptors produces an EPSP that is sufficient to depolarize the second order neuron to threshold. The release of glutamate from the pre-synaptic neuron can be modulated by a variety of pre-synaptic receptors such as cholinergic, adenosine, or neuropeptide receptors, demonstrating the complex regulation and modulation of synaptic connections.

Substance P (SP) is the second major excitatory neurotransmitter released from the central axonal terminal at the first synapse. SP is a short peptide that is a member of the tachykinin family of peptide neurotransmitters, and is derived from the pre-protachykinin-A (PPT-A)-gene (Hokfelt et al., 1975). This gene also codes for other tachykinins such as neurokinin A and neuropeptide K. Substance P is released by C-fiber nociceptor activation and binds to the specific tachykinin

receptors, called neurokinin-1 (NK_1) receptors. NK_1 receptors, along with substance P, are distributed in the brain and are specifically found in the hypothalamus, amygdala, and periaqueductal gray, regions that serve to regulate emotions, the autonomic nervous and endocrine systems, as well as nociception. In addition to its release from the central axonal terminals, substance P and other tachykinins are also released from the peripheral endings of nociceptors where they mediate effects called 'neurogenic inflammation' (Geppetti, 1996). By binding to the NK_1 receptor on vascular smooth muscle cells, substance P contributes to vasodilation and edema, a sign of localized inflammation (Laycock and Meeran, 2013).

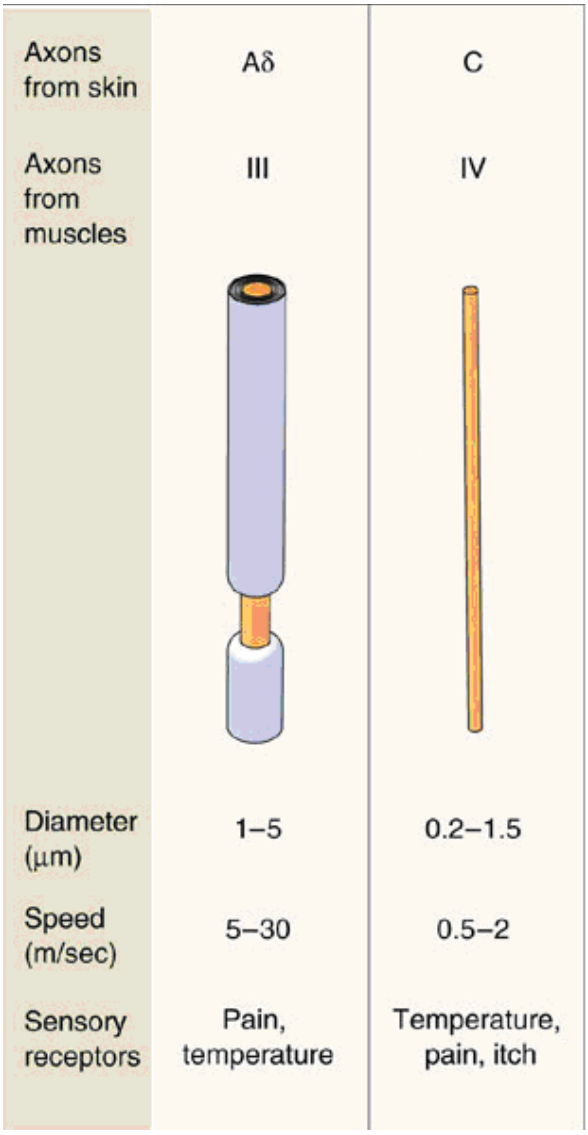

Table 1: Nociceptors

A-delta fiber nociceptors are thinly myelinated and are responsible for mechanical and thermal stimuli, carrying them quickly resulting in "fast pain." C-fiber nociceptors are unmyelinated and are responsible for the slow conduction of action potentials from mechanical, thermal, and chemical stimuli. (Table taken from Bear, 2007).

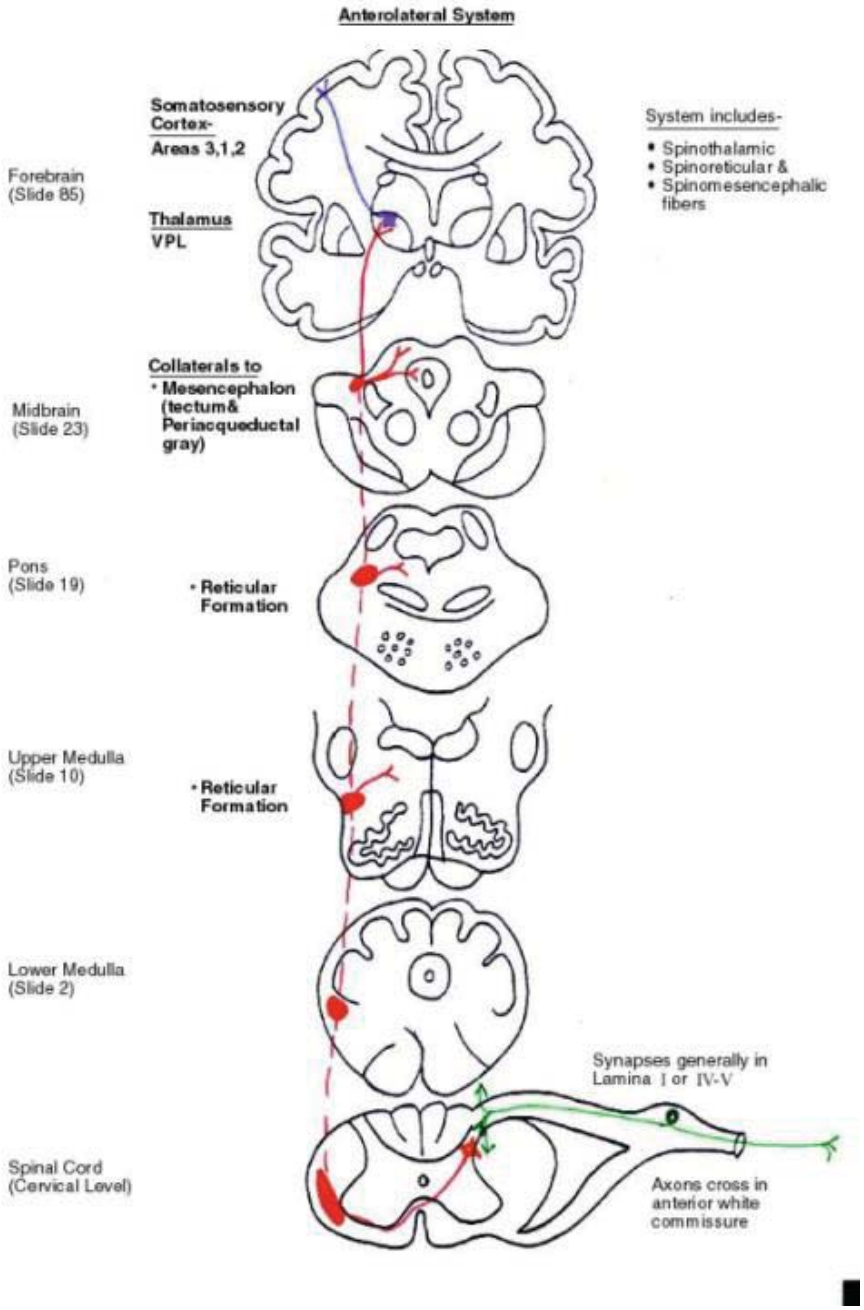

Figure 1: Anterolateral System (ALS)

The ALS is a group of sensory pathways ascending the spinal cord in the ante rolateral portion of the white matter that conveying information about tempera ture and noxious stimuli to the brain. The collateral terminations include the r eticular formation, thalamus, and somatosensory cortex.(Figure from the Inter active Neuroscience v3.0 Supplements).

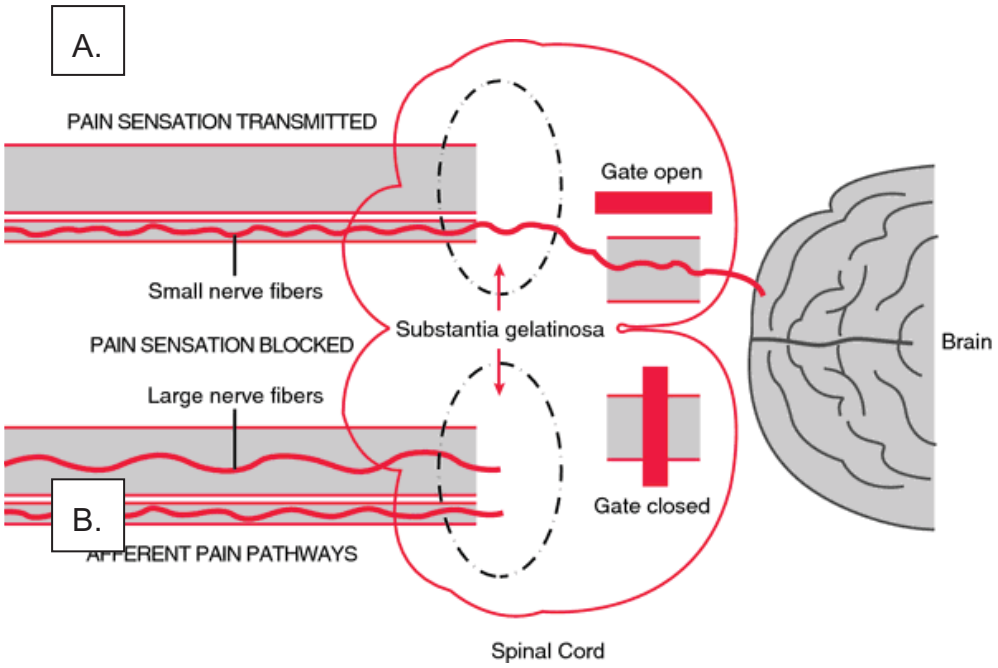

Figure 2: Gate Control Theory of Pain

C-fiber and A- delta nociceptors are represented by small nerve fibers above. A-beta fibers are represented by large nerve fibers above A) If an action potential is transmitted through the small fibers, the gate is "open," allowing the signal to travel to the brain.. B) If an action potential is transmitted through the large fibers, the gate is "closed," blocking nociception and no signal is sent to the brain. (Figure taken from Linton et al., 1999).

c. Dorsal Horn-Second Order Neuron

The first synapse in the transmission of pain information occurs within the dorsal horn of the spinal cord gray, between the nociceptive dorsal root ganglion neuron and a second order neuron. This region of the dorsal horn is called Rexed's lamina. Laminae I to IV are primarily associated with exteroceptive sensation, making these regions highly involved in the modulation and transmission of nociceptive stimuli within the CNS. Laminae I, called the marginal zone, is located at the superficial tip of the dorsal horn and receive information from thermal stimuli and non-noxious thermal stimuli. Lamina II, the substantia gelatinosa, is comprised of interneurons that receive sensory information. Lamina III contains neurons that mainly receive information about mechanical stimuli and lamina IV contains cells that receive information about light mechanical stimuli. Lamina V plays an important role in transmitting nociceptive sensations from visceral organs. The axon of the second order nociceptive neuron then decussates to the opposite side of the spinal cord, where it enters the anterolateral quadrant and ascends via the spinothalamic tract. This tract passes superiorly through the brain stem and terminates in the thalamus.

2. Thalamus- Third Order Neuron

The spinothalamic tract transmits information pertaining to noxious chemical, mechanical, and thermal stimuli to the thalamus. The thalamus is a subcortical area of the CNS known as the brain's relay station, and plays an important role in the relay of a variety of ascending sensory information, including nociceptive information, to the higher sensory processing centers of the cerebral cortex. Studies conducted on rats following hindpaw inflammation show that thalamic neurons have lowered thresholds to noxious mechanical and thermal stimuli due to sensitization brought about by the constant transmission of pain information, producing hyperalgesic responses

(Guilbaud et al., 1986; Ahmad, 2006). The nociceptive neurons of the spinothalamic tract terminate in the ventroposterolateral nucleus (VPL; Fig. 1) of the thalamus where they synapse onto third order thalamic neurons. These thalamic neurons then send their axons to the primary somatosensory cortex.

3. Primary Somatosensory Cortex

Nociceptive sensory input from the VPL of the thalamus terminates in the primary somatosensory cortex, located in the postcentral gyrus of the parietal lobe, which determines the location and intensity of a noxious stimulus (Bushnell et al., 1997; Kanda et al., 2000; Fig. 1).

The spinothalamic tract is not the only pathway responsible for the sensation of pain. There are several collateral pathways that branch from the spinothalamic tract that are responsible for the transmission of noxious input to accessory areas of the brain. These pathways have collectively been called the "pain matrix."

4. Pain Matrix

The term pain matrix was coined by Tracey and Manyth in 2007 as a means to describe the complexity of pain sensation. Rather than a simple, linear pathway from receptor to cortex, the idea of a "matrix" brings to mind a net of neuronal connections. The pain matrix is not a simple regulatory pathway, but is a collection of interconnected brain regions involved in cognition, emotion, motivation, and the sensation of pain (Tracey and Mantyh, 2007; Tracey and Johns, 2010). The pain matrix can be considered analogous to a train station, with the 3 neuron, ascending afferent pathway serving as the grand central station, or core, of the pain matrix. The areas that serve as this pain matrix "routing station" include the primary somatosensory cortex, insula, amygdala, and brain stem (Tracey and Mantyh, 2007).

The brain stem serves as an area of afferent as well as efferent nociceptive-

related signals in the pain matrix (Fig. 1). The spinoreticular tract brings afferent nociceptive information to the reticular formation of the brainstem and allows it to be processed without synapsing in the thalamus. Collateral afferent pathways of the pain matrix also target the rostral ventromedial medulla (RVM) nuclei of the medulla oblongata, the periaqueductal gray (PAG) in the midbrain, the locus coeruleus (LC) in the midbrain, and the nucleus raphe magnus (NRM) in the medulla oblongata. These areas contribute to the descending, efferent pain suppression pathway, inhibiting or modulating incoming nociceptive information at the spinal cord level (Ossipov, 2010).

Activation of the ascending nociceptive pathway elicits a simultaneous response from the descending nociceptive pathway. This pathway is an endogenous system that modulates the transmission of pain. The brain receives sensory input, as well as interprets these signals and makes constant adjustments in lieu of the changing conditions. The descending nociceptive pathway begins in the periaqueductal gray (PAG) located within the midbrain, an area with many opioid receptors and rich in endogenous opioid peptides, and projects to the medulla oblongata. The neurons that begin within the PAG produce an excitatory influence on neurons located within the RVM. The RVM contains the nucleus raphe magnus (NRM) which is abundant in serotonin. These serotonergic neurons act to inhibit action potentials carrying nociceptive information in order to reduce the transmission of pain. They do so by descending within the spinal cord and terminating in the dorsal horn where they activate enkephalinergic interneurons, causing the release of endogenous opioids. These endogenous opioids produce presynaptic inhibition of the primary afferent neuron by reducing their release of neurotransmitters and postsynaptic inhibition of the second order neuron, causing in a K^+ efflux and

inhibitory postsynaptic potential (IPSP). This IPSP, in turn, causes the release of endogenous serotonin and norepinephrine from axons that originate in the thalamus, reticular formation, midbrain, and spinal cord which acts to "block" pain signals by decreasing the action potential frequency within the spinothalamic tract (Vanegas et al., 2004).

While the descending pathways are activated to begin suppression of nociceptive sensations, afferent collateral pathways are also sending information to emotional areas within the CNS. Emotional pathways are those pathways responsible for taking pure nociceptive responses and storing them as the pain experience. It must be remembered that pain is a network phenomenon that is mediated, not by a single protein or brain region, but by multiple pathways at cortical, subcortical, spinal, and peripheral levels. These regions involve branches of neurons that lead to sites within the brain that store emotional memory and are involved in learning, including the amygdala and the secondary somatosensory cortex. These pathways are multisynaptic and pass through the reticular formation of the brainstem, allowing for the awareness of the stimulus to be identified. Once the nociceptive information is sent to the brainstem, it synapses in the central nucleus of the amygdala ("nociceptive amygdala"). The amygdala specializes in processing emotion and is essential for the expression of fear and anxiety, especially situations involving noxious stimuli (Phelps, 2004), and is active in the first stage of memory encoding. It can clearly be seen that the structures of the pain matrix play a vital function in the central processing of incoming signals and result in the conscious perception of a painful event.

C. Hypothalamic-Pituitary-Adrenal Axis (HPA-axis)

The pain matrix is activated not only during physical pain, but also in times of

stress, via the hypothalamic-pituitary-adrenal axis (HPA axis). The HPA axis is involved in the regulation of glucocorticoid stress hormones within the body. It is composed of the hypothalamus, the anterior pituitary gland, and the adrenal cortex of the adrenal glands. Stress can be both adaptive and maladaptive (Chapman, 2008), serving as a survival mechanism for an animal to escape a threat or actually hindering that animal in the time of threat. This axis consists of direct influences and feedback between the hypothalamus, pituitary gland, and adrenal gland, demonstrating an anatomical link as well as physiological link between the nervous and endocrine systems.

Within the hypothalamus lies the paraventricular nucleus (PVN) which produces corticotropin releasing hormone (CRH) that initiates the stress response. Nociceptive signaling acts directly on the PVN in order to increase CRH levels within the body (Chapmann, 2008). CRH is released into the median eminence, and then carried by the hypothalamic-hypophyseal portal circulation to the anterior pituitary, where it stimulates the release of adrenocorticotropin releasing hormone (ACTH). ACTH is then released into systemic circulation where it travels to the adrenal glands and stimulates the release of cortisol.

The main effects of cortisol generally last for a few hours and include increased blood pressure, increased glucose levels, suppression of the immune system, and decreased activity of the sympathetic nervous system which helps the body regain a state of 'normality' (Sapolsky, 1998). Cortisol binds to glucocorticoid receptors that are distributed in almost every cell of the body. Metabolic effects of cortisol include elevating blood glucose levels by stimulating glycogenolysis and counteracting the effects of insulin. Cortisol also works to regulate levels of electrolytes such as Na^+ and K^+. If the body needs Na^+ to be conserved, cortisol has

the intestines increases absorption of Na^+ by inducing vasodilation of afferent arterioles (Laycock, 2013). Normally, cortisol acts via negative feedback at the PVN level. Excess activation/stimulation of the PVN can cause a dysregulation, or abnormalitiy, of the HPA axis (Shepherd, 1994). If the HPA axis is no longer able to return to normal functioning following a stressful or painful situation, cortisol is continually produced and the hypothalamus and anterior pituitary become desensitized to potentially harmful situations.

D. Environmental Enrichment

Environmental enrichment (EE) is a broad area of research, with the core focus on understanding the effects of environmental variables on captive and research animals. The data collected from EE studies have wide-ranging implications, from improving animal welfare to applications in clinical biomedicine. Research into enriched environments has been conducted since the 1940's with the positive effects of EE on animal learning and memory introduced by Hebb. Hebb allowed his laboratory rats to freely roam throughout his house and observed improved behaviors when compared to rats housed in a standard laboratory environment (Hebb, 1947).

Since then, many studies have confirmed that rats housed in EE show improved learning and memory (Young, 1999). It has also been shown that being exposed to EE induces biochemical and structural changes in many brain regions, such as the hippocampus, which shows an increase in neuroplasticity (Diamond et al., 1976; Kempermann et al., 1997; Nithianantharajah and Hannan, 2006 and van Praag et al., 2000).

Recent studies concerning environmental enrichment have shown a decrease of stress in animals housed in an enriched environment when compared to those housed in a standard environment (Arco, 2007; Gabriel, 2010). Also, a decrease in

the degree of thermal hyperalgesia in those rats exposed to EE has been seen, suggesting that EE has a significant effect on nociceptive responses (Tall, 2009).

Environmental enrichment affects a range of physiological and behavioral effects on animals (Young, 2003). Regulatory agencies in the United States have been trying to conduct a movement to include physical enrichments as standard protocol for housing environments, rather than an as optional supplement (Hutchinson, 2005). There are certain regulations that need to be met under Federal Law when housing research animals in order to insure the animals' well-being. Currently, there are no mandates that EE must be provided in a laboratory by the Animal Welfare Act (AWA), however there have been many legal changes concerning EE as the primary housing condition for rodents, such as in Alternative Research and Development Foundation v. Veneman (Hutchinson, 2005). During this case, the Alternative Research and Development Foundation filed a petition for rulemaking, requesting that the term "animal" as defined in the AWA include birds, mice, and rats bred for the use in research. It was suggested that these animals be housed in a "natural" environment, taking into consideration the animals' structural, social, and activity needs (NRC, 1996).

Although the founding research in EE was conducted in rodents, the physical enrichment of animals such as horses and pigs has produced interesting findings. First, horses that were exposed to a pasture all day, every day (enriched group) showed the drive to exercise and graze more willingly than those horses that stayed in a pasture for a limited amount of time (control group; Houpt et al., 2001; Christensen et al., 2002; Chaya et al., 2006). The enriched horses also displayed a more varied rolling behavior, interpreted as a sign of comfort (Hansen et al., 2007). Pigs placed in an enriched environment displayed anticipation and "play" as measured through

observation of rooting around and head-butting cage mates. Also, providing visual barriers by placing boards or hay bales in the cages allowed the pigs to have a place to hide their heads (a target of aggression) in order to avoid aggressive pen-mates (Stolba and Wood-Gush, 1984; McGlone and Curtis, 1985; Fraser et al., 1986; Pedersen et al., 1993; Waran and Broom, 1993; Anderson et al., 1999).

From the perspective of Federal Law, EE is a husbandry issue, but to a preclinical biomedical researcher, EE can become a distinct cohort in a study. EE can be divided into five categories based on the subtype of enrichment provided. The first category is social enrichment, consisting of animals being in contact directly or indirectly with animals of the same species or humans (Bloomsmith et al., 1991). The second category is known as occupational enrichment, including devices that provide animals with control or challenges and promotes exercise. The third category is physical enrichment, including alterations of home cage size and inclusion of objects. The fourth category is sensory enrichment that includes visual, olfactory, tactile, auditory, or taste stimuli given at testing times in the home cage. The last category is nutritional enrichment, including changes in the nutritional content provided to the animal (Bloomsmith et al., 1991). In the present study, EE served as a treatment, specifically physical EE and its effects on nociception.

1. Preclinical Environmental Enrichment - Nociception

The use of animals in the studies of environment and nociception has played an important role in helping researchers to elucidate the relationships between the external environment and pain sensation. Many studies have shown that morphological and biochemical indices in the brain undergo alterations in response to the influence of the environment (Ickes et al., 2000). The brains of animals housed in EE show an increase in synapse number on pyramidal neurons, the main projecting or

output neurons from the cerebral cortex (Pham et al., 1999). With an increase in the number of synapses present, there is an increase in synaptic activity as well as an increase in size and number of the glial cells used for support. The above findings could be directly related to how EE affects nociception. Once an action potential is produced, it will travel to every presynaptic axon ending in that neuron and if more synapses are produced as a result of EE, alternative pathways are now present for the transmission of pain information. Since sensitization is a result of repeated stimulation, the additional synapses allow for a reduced level of activity in each individual synapse, resulting in a prevention of sensitization and reduced hyperalgesia and allodynia.

Along with producing anatomical changes within the CNS, EE also has anxiolytic effects. Stress can be either adaptive or maladaptive to an organism but some detrimental effects include behavioral/cognitive deficits and brain damage (atrophy).The majority of preclinical studies of EE and stress have shown that EE serves to temper animals' emotional reactivity to stressors (Chamove, 1989). The benefits of EE for combating stress can best be seen with hippocampal neurogenesis. Studies have supported that the brain's ability to adapt and change in given situations, by creating new pathways, could be a direct effect of EE (Kempermann, 2006). The positive preclinical findings of EE have clinical applications. However there is, limited data demonstrating physical and psychological benefits of EE in humans.

2. Clinical Environmental Enrichment - Pain

Clinical studies have shown that pain is not only a result from nociception, but the sensation of pain is also strongly influenced by emotional and social factors. A study done by Ulrich (1984) supports the hypothesis that changes in an environment can affect a person's sensation of pain. This retrospective analysis compared patients

with a view through a postoperative hospital room window of a natural scene versus a brick wall. The patients who were in the rooms with the natural scenes required less pain medication and were discharged from the hospital more rapidly, suggesting that external environmental variables affect pain. With slight data showing EE has positively affected nociception, the question arises if the benefit of EE was due to pre-pain or post-pain environment.

E. Cross-Over Research Studies

Common in clinical investigations, cross-over studies include patients or human volunteers receiving a prescribed sequence of treatments, then at a set point in the investigation a proportion of the subjects "cross" to the other treatment. This type of study uses fewer patients than what would normally be required because the patients are able to serve as their own control. A cross-over study begins with two cohorts: a control group (AA) and an experimental group (BB). At a particular point in the study a cross-over will occur and four cohorts will proceed in the subsequent investigations; AA, AB, BB, BA.

There is a paucity of data on the effect of EE on nociception in animals and a lack of data examining cross-over of home cage environments. Based on previous work done in this lab (Tall, 2009) on enriched environments alone, it can be hypothesized that EE rats should be less affected by nociceptive stimuli than rats in standard home cage environments. The current investigation expanded on previous work from this laboratory examining the effects of home cage environment on nociceptive behavior by looking at home cage environment prior to and following experimentally-induced inflammation. What would happen if a cross-over cohort study was conducted; would the same results as seen by tests previously conducted in this lab hold true if rats were raised in an enriched environment then switched to a

standard environment once experimental nociception was induced? What if rats raised in a standard environment were switched to an enriched environment after induced inflammation? It was hypothesized that subjects in enriched housing condition throughout the entire experiments would exhibit the highest thresholds to mechanical and thermal stimuli after inflammation, indicating an analgesic effect of enrichment. This group would be followed by the standard-enriched, showing the second highest, the enriched-standard showing the second lowest, and the standard-standard showing the absolute lowest thresholds after inflammation was induced.

Chapter 2

II. Methods

A. Animals

Studies were performed on male Sprague-Dawley rats 27-32 days old upon arrival, obtained from Charles River Laboratories Inc. (Wilmington, MA). Four groups of rats were used with n=6 per housing condition, for a total of 24 rats. Rats were maintained on a 12/12 light/dark cycle, with lights off at 10:00 a.m. and lights on at 10:00 p.m. The animal facility and behavioral testing laboratory was maintained at 21-22 degrees Celsius. Food (Lab Diet 5P00 Prolab RMH 3000 PMI Nutrition International Brentwood, MO) and water were provided ad libitum. Fresh bedding (aspen pine shavings and Bed-O-Cobb) was provided during the weekly cage change/cleaning by the animal facility staff.

B. Housing Conditions

Animals were randomly placed, by the animal facility staff, in polycarbonate cages measuring 20" long x 16" wide x 8 1/2" tall upon arrival (N=3 rats per cage). Food, water, and bedding were included in all home cage housing conditions. After one week of habituation, the rats were randomly assigned a housing environment by cage; meaning, 12 rats (4 cages) were assigned as standard, and the remaining 12 rats (4 cages) were assigned as enriched or supplemented (Fig. 4). The physically supplemented cages included nylabones (4.5" x 1.25"), rat tunnels (round and square), crawl balls, barbells, and chew toys that were changed during the cage changing each week. After a five week pre-treatment period, the cross-over occurred (rats were 62-67 days old); 6 of the 12 rats in the standard cages remained standard while the remaining 6 were crossed to enriched housing. Six of the original 12 enriched remained enriched while the remaining 6 were crossed to standard housing.

A representation of each housing condition is shown in Figure 3.

A.)

B.)

Figure 3: Housing Conditions

A.) A standard housing cage supplied with food, water, and bedding. B.) An enriched housing cage supplied with various toys, food, water, and bedding.

C. Inflammation Model

Following the 5 week housing pre-treatments, while under brief isoflurane anesthesia (3%), an intraplantar injection of 100 microliters of 100% Complete Freund's Adjuvant (CFA) was administered into the left hind paw on the plantar surface, between the foot pads (Stein, 1988; Fig. 4).

D. Edema Quantification

Thickness measurements of the dorso-ventral hind paw were made using a caliper in order to assess the degree of edema in the CFA-injected hind paw. Baseline data were first collected before the injection of CFA and post-inflammation edema data were collected at multiple points after the injection (2h, 7d, 14d, 21d, 28d, and 35d; Fig. 4).

E. Behavioral Assessments

Animals were habituated to the behavioral testing laboratory, testing equipment and investigators for 2 hours, 3 days a week for 1 week. Baseline measurements were collected subsequent to the habituation and animals' weight was collected each week. Post inflammation behavioral data were collected at multiple times after injection (2h, 7d, 21d, 28d, and 35d). All testing was performed between 10:00 a.m. and 3:00 p.m., during the dark phase.

F. Paw Withdrawal Threshold (PWT) to a Mechanical Stimulus

The withdrawal threshold to a mechanical stimuli was measured using a set of nine calibrated von Frey monofilaments (0.2-32.0 grams force) using the Up-Down Technique (Chaplan et al., 1994; Dixon, 1980). Rats were placed in plexiglass chambers on an elevated stainless steel mesh screen and allowed 5-10 minutes to habituate. The von Frey monofilaments were applied for 5 seconds with a small amount of pressure to cause a buckling of the monofilament against the plantar

surface of each hind paw. A positive withdrawal was considered as a raising or licking of the hind paw.

G. Paw Withdrawal Latency (PWL) to a Heat Stimulus

Paw withdrawal from a heat stimulus was tested using the Hargreaves method (Hargreaves et al., 1988). Rats were placed on a glass table in plexiglass chambers and were allowed to habituate for 5-10 minutes. In order to quantify paw withdrawal latency, a heat source was focused on each hind paw in the area between the pads. Each hind paw, CFA injected and non-injected, was tested four times with at least 5 minutes between successive application of the radiant heat source in order to avoid sensitization. The PWL of each rat was calculated as a mean of trials 2-4. A positive withdrawal was considered as a raising or licking of the hind paw.

H. Statistical Analysis

The difference scores for paw thickness, PWL, and PWT measurements were calculated by subtracting the right paw measurement from the left paw measurement, allowing the right paw to serve for the control for each animal. The data were then expressed as the standard error of the mean (S.E.M.) and analyzed using a repeated measure analysis of variance (ANOVA) in IBM SPSS Statistics version 20. A p value of < 0.05 was considered statistically significant

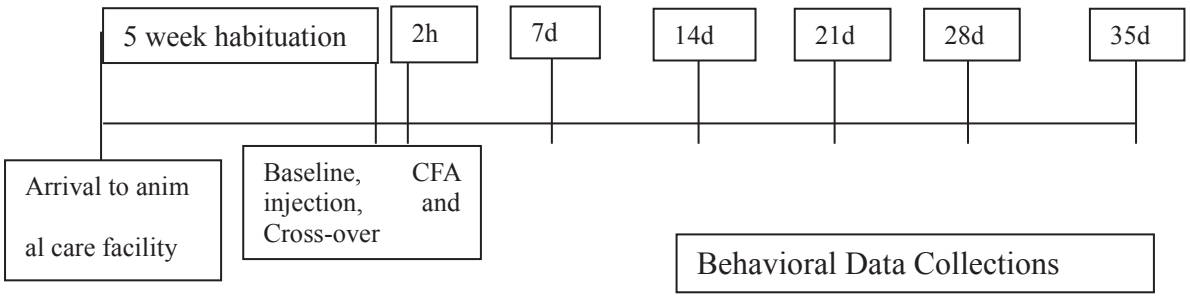

Figure 4: Experimental Design

The line graph above depicts the timeline each group of rats followed upon arrival to the final testing day. Following habituation, h=hours and d=days.

Chapter 3

III. Results

A. Paw Thickness

Paw thickness was measured using a caliper in order to assess the relative degree of inflammation induced from the injection of CFA. It was demonstrated that at 2 hours post-CFA injection, standard-standard showed the thickest paws, followed by standard-enriched, enriched-enriched, and enriched-standard. At 7 days post, all four housing conditions showed an increase in thickness when compared to 2 hours. Thickness values for all four housing conditions were seen to continue to decrease each week until 35 days post-CFA injection (Fig. 5).

A repeated measures ANOVA was conducted on paw thickness for all four treatment groups (standard-standard, standard-enriched, enriched-standard, enriched-enriched) at baseline, 2 hours, 7 days, 14 days, 21 days, 28 days, and 35 days post-inflammation. There was no significant difference among home cage treatment groups (p-value = 0.180; $F_{10.125,44}$=1.403). There was, however, a significant difference across testing times (2h, 7d, 14d, etc.; p-value < 0.001; $F_{3.485,44}$=1.403).

B. Paw Withdrawal Threshold (PWT)

Animals were tested for PWT to mechanical stimuli using a set of nine calibrated von Frey monofilaments (0.2-32.0 grams force) with the Up-Down Technique (Chaplan et al., 1994; Dixon, 1980). This method was used in order to determine if an enriched environment affects mechanical-related nociceptive behavior. At 2 hours post-CFA injection standard-standard, standard-enriched, and enriched-standard groups all showed a significant decrease in PWT values from baseline, while the enriched-enriched group showed a slight decrease. Values recorded at 7 days post injection showed an increase in threshold for the standard-

standard group, a slight decrease for the standard-enriched group, a decrease for the enriched-standard group, and a significant decrease for the enriched-enriched group. The threshold values recorded at 14 days and 21 days post stayed relatively the same as the data recorded at 7 days for each group. The standard-standard and enriched-standard groups at 28 days showed an increase in threshold from 21 days. Standard-enriched and enriched-enriched groups showed a significant increase in threshold value. At 35 days post-CFA injection, standard-standard showed a significant increase in threshold value while the other three housing groups showed significant decreases (Fig. 6)

The repeated measures ANOVA conducted on PWT revealed and no significant difference among the four housing groups (p-value = 0.311; $F_{3.801,44}$ = 2.520). There was a slight significant difference across behavioral testing times (p-value = 0.046; $F_{11.402,44}$=1.169),.

C. **Paw Withdrawal Latency (PWL)**

Animals were tested for PWL from a heat stimulus using the Hargreaves method (Hargreaves et al., 1988). This method was used in order to determine if an enriched environment affects thermal-related nociceptive behavior. PWL values recorded at 2 hours post-CFA injections were seen to be significantly lower than those collected at baseline. At 7 days, standard-standard, enriched-standard, and enriched-enriched showed slight increases from 2 hours while standard-enriched showed a slight decrease. All four housing groups showed a significant increase in PWL at 14 days. At 21 days, standard-standard and enriched-standard showed slight increases, standard-enriched showed a slight decrease, and enriched-enriched showed a significant increase. PWL values remained similar to those recorded at 21 days until 35 days (Fig.7).

The repeated measures ANOVA conducted on PWL showed no significant difference

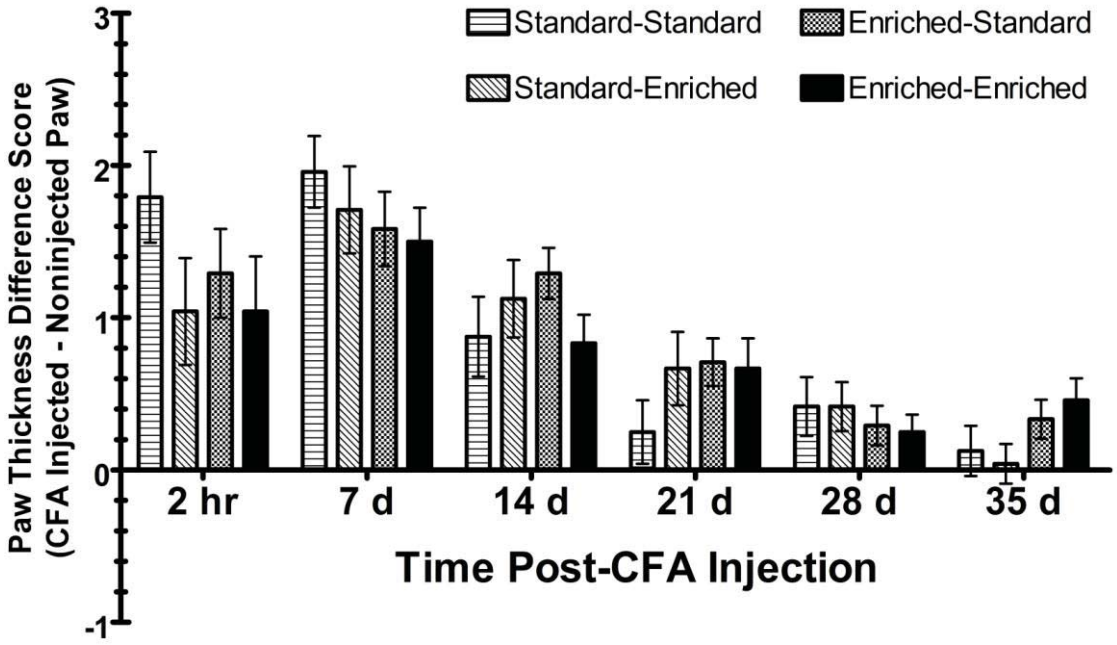

Figure 5: Paw Thickness Measurements

This figure shows a comparison of rats housed in standard/standard, standard/enriched, enriched/standard, and enriched/enriched cages. Thickness was found using calipers. Data is presented for treatment groups at post 2 hours, 7 days, 14 days, 21 days, 28 days and 35 days. Values are represented as the difference score of the S.E.M. Values were analyzed using a repeated measure ANOVA, which revealed $F_{10.125,44}$=1.403 with no significant difference among home cage treatment groups (p-value = 0.180). There was, however, a significant difference across testing times (2h, 7d, 14d, etc.; p-value < 0.001) and $F_{3.485,44}$=1.403.

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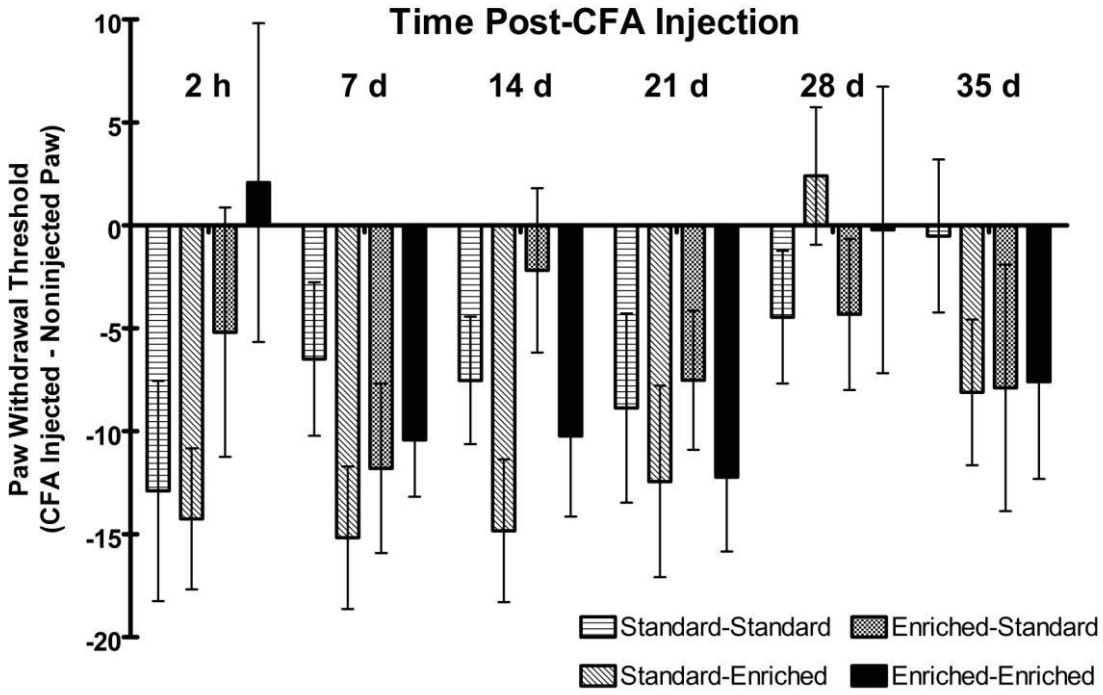

Figure 6: PWT Measurements

This figure shows a comparison of rats housed in standard/standard, standard/enriched, enriched/standard, and enriched/enriched cages. PWT was found using von Frey monofilaments to perform the Up-Down technique. Data is presented for treatment groups at baseline and post 2 hours, 7 days, 14 days, 21 days, 28 days and 35 days. Values are represented as the difference score of the S.E.M. Values were analyzed using a repeated measure ANOVA, revealing revealed $F_{3.801,44} = 2.520$ and no significant difference among the four housing groups (p-value = 0.311). There was a slight significant difference across behavioral testing times (p-value = 0.046), and $F_{11.402,44}=1.169$.

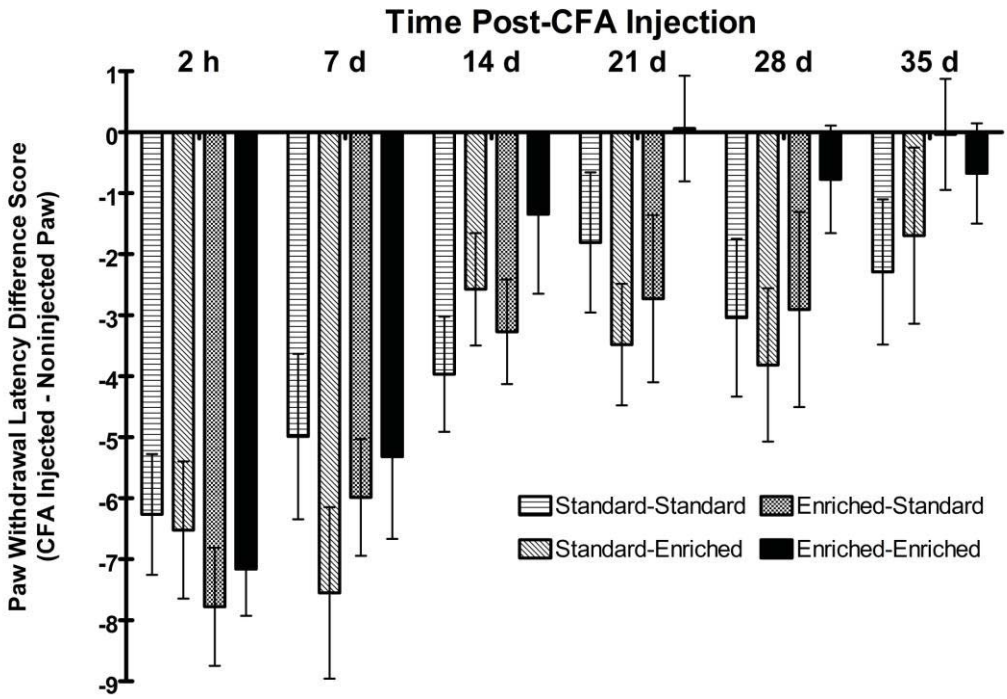

Figure 7: PWL Measurements

This figure shows a comparison of rats housed in standard/standard, standard/enriched, enriched/standard, and enriched/enriched cages. PWL was found Hargreaves method. Data is presented for treatment groups at baseline and post 2 hours, 7 days, 14 days, 21 days, 28 days and 35 days. Values are represented as the difference score of the S.E.M. Values were analyzed using a repeated measure ANOVA, which revealed no significant difference among home cage housing groups (p-value = 0.446) and $F_{11.244,44}$=1.004.There was a significant difference across behavioral testing times (p-value < 0.001) and $F_{3.748,44}$=22.405.

among home cage housing groups (p-value = 0.446) and $F_{11.244,44}$=1.004.There was a significant difference across behavioral testing times (p-value < 0.001) and $F_{3.748,44}$=22.405.

Chapter 4

V. Discussion

The present study examined the effects an environmental cross-over on inflammation-induced nociception. In this investigation, rats were reared in two distinct housing treatment groups prior to the establishment of a model of experimental inflammation. Following the onset of sub-chronic inflammatory response, half of the rats were crossed to the other environment, while the other half stayed in the same environment. Prior to collecting data on inflammation - induced nociception, Thickness, PWT and PWL data were collected and served as a baseline point of comparison. After five weeks of living in their initial home cage environments, subjects were injected with CFA into their left hind paw to induce inflammation. The subjects were then crossed-over into their post inflammation environments and remained there for 35 days,with post inflammation data being collected once a week.

In support of previous work, paw thickness, a tool to quantify the degree of inflammation, showed a significant difference across behavioral testing times (Fig.5). This demonstrates that the injection of CFA produced a localized inflammation in the hind paw. A significant difference among housing treatment groups was not found, suggesting that the severity of inflammation produced from an intraplantar CFA injection is not affected by the physical home cage environment. This finding supports previous work done in this laboratory (Tall, 2009) and work done by K. Militzer (Militzer, 1975), along with almost all other studies done using CFA to produce localized inflammation within the hindpaw.

According to the literature, PWT is not affected by environmental conditions (Tall, 2009). This was supported by the present study, as no significant difference

among housing groups was found. There was, however, a slight significant difference seen across behavioral collection times (Fig. 6). These data indicate that there is a difference in mechanical and thermals hyperalgesia, which was not unexpected (Meller, 1994 and Tall, 2009).

PWL to a radiant thermal stimulus showed no significant difference across behavioral testing times, not in support of PWL being affected by an intraplantar CFA injection (Fig. 7). A significant reduction in withdrawal latency was shown across all four housing conditions, but there was no significant difference among the housing treatment group. This finding suggests that PWL is not affected by the physical home cage environment. This finding does not support many other studies done on PWL (Hargreaves, 1988; Dubner, 1983; Schneider, 2006). It does, though, refute previous work done in this lab (Tall, 2009).

When looking at Fig. 6, a few trends can be seen. First, looking at the standard-standard housing environment, PWT values were increased greatly from baseline (0). The values slightly decreased and increased over the course of data collection, but by day 35 post, standard-standard revealed the lowest PWT difference values, meaning a high PWT. It could be speculated that being raised in a standard environment and staying in a standard environment after localized inflammation was introduced could allow for a faster recovery when exposed to mechanical stimuli because no additional stress was introduced by the changing of an environment. As mentioned previously, stress can be either positive or negative. When relating to the crossover to a new environment, stress would be considered to have negative effect on the body, causing excess activation of the HPA-axis and/or nociceptive pathways (Chapman, 2008). When comparing the other three housing environments (standard-enriched, enriched-standard, enriched-enriched) values were seen to be very sporadic

with no identifiable trend, as expected from previous studies.

Difference values for PWL (Fig. 7) show an evident trend. Standard-standard was seen to have the highest PWL thresholds from 2 hours post to 7 days post injection. Animals in this housing condition were then seen to have a slowed recovery, resulting in standard-standard having the lowest thresholds at 35 days post. It could be speculated that the animals in standard-standard were subjects of sensitization of the nociceptive pathway, resulting in lower activation thresholds and increased activation of pain transmission, as mentioned previously. The standard-enriched showed a similar pattern as the standard-standard housing condition, suggesting that being reared in a standard environment at a young age leads to excessive activation of synapses, resulting in sensitization, when placed in a stressful/painful situation (Larsson, 2002; Baroncelli, 2010; Fox, 2006; Benaroya-Milshtein, 2004). The enriched-standard environment showed the lowest PWL threshold at 2 hours post CFA injection, but rapidly being to increase as the study progressed. By day 35 post, enriched-standard revealed the highest values. The enriched-enriched environment showed a rapid increase in PWL threshold until 21 days post. At 28 days post, although values increased from the previous week, enriched-enriched still showed the highest threshold. The data collected from these two housing conditions once again suggest that the initial housing environment plays a bigger role than the post inflammation housing environment. Being reared in an enriched environment allows for more synapses to be developed, thus reducing sensitization in synapses within the nociceptive pathway (Larsson, 2002; Baroncelli, 2010; Fox, 2006; Benaroya-Milshtein, 2004; Diamond et al., 1976; Kempermann et al., 1997; Nithianantharajah and Hannan, 2006). Pain information now has more pathways to travel through, lowering the activation frequency of individual synapses,

"exhausting" them slower.

In future studies, improvement and further examination could be made on some variables. Increasing the number of pre-treatment data recorded after the 5 week habituation would allow researchers to have more data to compare post-treatments to, rather than just a single baseline. This would also determine whether or not housing has an effect on non-injured rodents. Also, extending the amount of time data is collected post-inflammation could be beneficial, allowing more time for recovery. The enriched-enriched group for PWL at post 35 days shows the threshold still increasing. By increasing the amount of post-inflammation time points, it could be determined how long it takes for the thresholds to level out or stay steady for each housing condition. In previous studies performed in this lab, it was shown that home cage numbers (socially vs. isolated) has an effect of nociceptive thresholds. Socially housed animals would include three animals per cage, while housing isolated would be a single animal per cage. It could be beneficial to conduct a cross-over study using isolated versus socially housed subjects. These data would elucidate if social housing and cross-over study affects inflammation-induced nociception. A few of the limitations of this study include only using male rats and starting with young rats. Using various ages would determine if the same results would be shown with rodents that were adult throughout the whole experiment.

Although this study did not support that crossing over from one environment to another significantly affects inflammation induced nociception, it is important to remember that being reared in an enriched environment does show significant improvements in nociceptive thresholds when compared to being reared in a standard environment (Hutchinson, 2005). When an animal is housed in an environment that allows them to exhibit natural behaviors, "less" pain is felt when subjected to

research. With this being said, EE is becoming a key standard when housing laboratory animals because it improves animal welfare overall (Hutchinson, 2005).

Chapter 5

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