Anatomical Specificity of Acidic Saline Model of Chronic Pain and the Role of Glia in Development of Chronic Hyperalgesia

By

Lisa Jasper BSc BMR(PT)

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ABSTRACT

Research into mechanisms of hyperalgesia is ongoing with the goal of improving the clinical management of chronic pain. Although several animal models of chronic pain are reported in the literature, few address painful muscle conditions such as fibromyalgia. One such model recently developed in Sprague-Dawley rats uses two acidic saline injections in a gastrocnemius muscle five days apart to induce a long-lasting change in bilateral paw withdrawal thresholds. The first objective of this study was to determine if the two injections needed to occur in the same muscle. Paw withdrawal thresholds were measured by applying von Frey filaments to the plantar surface of the hindpaw; the development of hyperalgesia was indicated by a decrease in paw withdrawal threshold. Acidic saline injections were administered in either the right lateral, right medial or left lateral gastrocnemius muscle. All animals received a second injection in the right lateral gastrocnemius muscle. Paw withdrawal thresholds decreased bilaterally in all animal groups demonstrating that hyperalgesia still develops when the site of the first injection is varied. Additionally, animals in which the first muscle injection was substituted with a non-specific treatment (intraperitoneal injection of lipopolysaccharide) also developed bilateral hyperalgesia. These results demonstrate that the mechanism of chronic pain in this model lies outside of the injected muscles and may be mediated primarily by central nervous system structures. Given the role of central glia cells in other pain models it was next assessed whether the development of hyperalgesia could be blocked by pretreatment with minocycline, an inhibitor of glia cell activation. Pretreatment with minocycline prior to the first muscle injection prevented the development of hyperalgesia whereas minocycline was ineffective when administered before the second muscle injection. These data indicate that central processes including glia cell activation play important roles in the development of hyperalgesia in this model of chronic muscle pain and provide a potential target in the development of interventions (physical and pharmacological) aimed at chronic muscle pain.

ACKNOWLEDGEMENTS

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INTRODUCTION

Chronic pain is one of the most intriguing and challenging areas of clinical practice and research. Its presentation is as varied as the individuals experiencing it. The onset may correspond to a specific precipitating event such as a whiplash injury or be insidious with diffuse symptoms such as fibromyalgia. Many people with chronic pain show an increased sensitivity to painful stimuli (hyperalgesia) and perceive formerly pain free stimuli as painful (allodynia). Unfortunately, most cases are similar in their difficulty to assess, quantify and treat effectively. The cost of chronic pain to society is staggering when one considers the loss in productivity, sick days, health care costs and, perhaps most significantly, the cost to each individual's quality of life. Our current health care model is far more successful at treating acute pain than chronic pain despite a recent increase in research with a chronic pain focus. Research into the mechanisms of hyperalgesia is ongoing with the goal of improving the clinical management of chronic pain. Many models are used in investigating mechanisms of pain; however, there are few models of pain that are chronic and muscle-based even though this is a common clinical scenario. Sluka et al developed a model of chronic musculoskeletal pain using repeated injections of acidic saline in rats (Sluka, Kalra, & Moore, 2001). This investigation has further characterized the model by varying the site and nature of the first acidic saline injection as well as investigating the role of glial cells through use of a glial inhibitor.

REVIEW OF LITERATURE

Hyperalgesia

At its most basic functional level, pain serves as an important warning system for noxious stimuli that may cause tissue damage. When a noxious stimulus is sensed, nociceptors are activated transmitting the message to the dorsal horn of the spinal cord and then to higher central nervous system structures where the sensation of pain is perceived. However, if tissue injury does occur there is a transformation in pain processing. Hyperalgesia, which is an increased sensitivity to painful stimuli, or allodynia, the perception of formerly pain free stimuli as painful, may develop. The increase in pain sensitivity at the injury site is referred to as primary hyperalgesia. A further complexity of injury and the resulting pain, however, is the ability of the increased pain sensitivity to spread to uninjured sites, a process known as secondary hyperalgesia. This phenomenon may be restricted to a narrow border of uninjured tissue which immediately surrounds the injury site but may also cover a much more expansive area. One of the most fascinating types of secondary hyperalgesia is mirror pain in which the contralateral tissue also displays increased pain sensitivity in a symmetrical pattern despite the lack of any observable inflammatory or injury process in the contralateral tissue. The potential for hyperalgesia to develop also in a nonsegmental manner is indicated by clinical conditions such as fibromyalgia. While the precise mechanism responsible for generating secondary hyperalgesia is unknown, many current research efforts are focused at the spinal cord level.

The initial inflammatory response including bradykinin, histamine and prostaglandin release leads to vasodilation, endothelial cell contraction, neurotransmitter release and edema. An additional outcome is sensitization of the peripheral nociceptors which reduces the input required to activate the nociceptors. The nociceptors transmit the sensation of pain to the dorsal horn typically to Laminas I, II and V. Two main types of nociceptive fibres are A-delta and C fibres. A-delta fibres are small-diameter, fast-conducting myelinated fibres carrying sensations of sharp, well-localized pain. C fibres are small-diameter, slow-conducting unmyelinated fibres carrying sensations of dull, poorly localized pain. 10-20% of C fibres are normally silent or inactive but may become activated in an inflammatory response also contributing to the hyperalgesic response. Glutamate is the major excitatory neurotransmitter released by the nociceptors and acts upon NMDA and non-NMDA type receptors. If there is repetitive firing of C fibres, the dorsal horn neurons may increase their responsiveness which is a process called wind-up. This process involves glutamate and NMDA receptors as it is ceased if the NMDA receptors are blocked (McMahon, Lewin, & Wall, 1993). The dorsal horn neurons may be nociceptive-specific neurons or wide dynamic range neurons and transfer the information across the spinal cord to travel in the spinothalamic tract to the thalamus and finally to the sensorimotor cortex and other brain sites (for review see (Coutaux, Adam, Willer, & Le Bars, 2005)). Central sensitization refers to increased sensitivity or excitability of central nervous system structures including the spinal cord and supraspinal structures involved in pain processing.

A third type of sensory afferent fibre can also be involved in tissue injury. A-beta fibres are myelinated fibres which conduct information from touch receptors, normally non-nociceptive. These fibres are, however, implicated in allodynia through alterations in central processing (Koltzenburg, Lundberg, & Torebjork, 1992; Koltzenburg, Torebjork, & Wahren, 1994; Klede, Handwerker, & Schmelz, 2003)). For example, this was demonstrated in a human study using capsaicin and mustard oil as noxious stimuli where the resulting allodynia was reversed when a peripheral nerve block was done to selectively eliminate light touch sensation (Koltzenburg et al., 1992). Further, Torebjork et al demonstrated that electrical stimulation of non-nociceptive fibers resulted in pain sensations following capsaicin treatment of skin that was adjacent to, but outside of, the receptive field of the non-nociceptive fibers (Torebjork, Lundberg, & LaMotte, 1992).

Aside from known processes within neuronal cells, a distinct role in hyperalgesia for nonneuronal glial cells, especially microglia, is also emerging. Glial cells have long been known to have a supportive role to neurons, both separating and insulating them, removing debris and producing myelin. In the central nervous system, glia can be divided into macroglia (astrocytes and oligodendrocytes) and microglia. Astrocytes are the most numerous type of glial cells and are thought to have a role in maintaining homeostasis especially extracellular pH, ion and neurotransmitter concentrations. Microglia are phagocytes which become activated during injury or infection. Activated microglia are thicker and more branched than resting cells and are known to have increased expression of antigens such as OX-42 (Moalem & Tracey, 2006). Examples of pain models implicating a role for glial cells by showing activation through altered glial morphology include intraplantar injection of formalin (Fu, Light, Matsushima, & Maixner, 1999; Sweitzer, Colburn, Rutkowski, & DeLeo, 1999; Watkins, Martin, Ulrich, Tracey, & Maier, 1997), ligation of spinal nerve and nerve roots (Hashizume, DeLeo, Colburn, & Weinstein, 2000; Winkelstein, Rutkowski, Sweitzer, Pahl, & DeLeo, 2001) and complete Freund's adjuvant induced arthritis (Raghavendra, Tanga, & DeLeo, 2004). Glial inhibitors such as fluorocitrate and minocycline have provided further evidence for a glial role as they have been shown to prevent or decrease pain in many models (Watkins et al., 1997; Ledeboer et al., 2005; Milligan et al., 2003; Milligan et al., 2000). Bidirectional communication has been demonstrated between central neurons and glial cells with glutamate and NMDA receptors playing a key role (Verkhratsky & Kirchhoff, 2007). When activated, glial cells are known to express proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin-1 (IL-1) and interleukin-6 (IL-6) which may influence neurons as well as other glia. Pro-inflammatory cytokines have been shown to play a role in exaggerated pain states (Sweitzer, Martin, & DeLeo, 2001; Watkins et al., 1997). Spataro et al hypothesize that spinal gap junctions between glial cells may be involved in the spread of hyperalgesia as using the gap junction decoupler carbenoxolone stops the development of mirror pain while not affecting the ipsilateral mechanical allodynia (Spataro et al., 2004)

Identifying the mechanisms through which the hyperalgesia develops will hopefully allow insight into mechanisms of human chronic pain.

Acidic Saline Model

Chronic musculoskeletal pain is a major cause of disability in our society. However, when investigating mechanisms of hyperalgesia, there are few pain models that are chronic and muscle-based. Accordingly, Sluka et al developed a model of chronic pain using repeated injections of acidic saline in rats (Sluka et al., 2001). Two injections of pH 4.0 were given into one lateral gastrocnemius muscle on day 0 and day 5. Twenty-four hours after the second injection a significant bilateral decrease in mechanical withdrawal threshold using Von Frey filaments was found (see figure 1). Baseline withdrawal thresholds range between 160 mN and 250 mN which are typically reduced to 25-80 mN after induction of hyperalgesia (Sluka, personal communication). As reported by Sluka, the hyperalgesia persists for 4-5 weeks (Sluka et al., 2001). This finding forms the basis of the model of mechanical hyperalgesia.

Nielsen et al found approximately 70% of the animals used in the acidic saline model responded with the development of the mechanical hyperalgesia (Nielsen, 2004). This was consistent with results from Dr. Sluka's lab that developed the model (personal communication). This response rate must be considered when developing experimental group sizes.

Two manipulations were carried out to test for the necessity of continued primary afferent input. Lidocaine was injected into the gastrocnemius muscle after the second injection of acidic saline. They found no significant differences in the withdrawal threshold on the contralateral side with the lidocaine or a control saline injection. In the second manipulation, ipsilateral dorsal rhizotomies were performed 24 hours after the second injection. The mechanical withdrawal thresholds on the contralateral limb remained decreased similar to the values measured prior to the dorsal rhizotomy again providing evidence against the role of continuing primary afferent input (Sluka et al., 2001).

Observation and a treadmill test were used to determine there were no significant changes in the motor ability of the animals throughout the testing. Observations included no limb guarding, equal weight bearing, normal gait patterns and normal placing reflex. Upon analysis of muscle histology, no damage to the muscle tissue was evident after either injection in the majority of the animals (Sluka et al., 2001).

The effects of timing between injections were tested finding there was an equivalent decrease in the mechanical withdrawal threshold when the injections were either 2 or 5 days apart but no change in the mechanical withdrawal threshold when the injections were 10 days apart(Sluka et al., 2001). It is interesting that the model requires the second injection to follow within 2-5 days of the first raising the possibility of a critical timeframe in which hyperalgesia is more likely to develop. This raises the question of what activation in the process of hyperalgesia is occurring that persists for a limited time period and then dissipates.

Sluka et al found the model dependent on peripheral changes in pH as hyperalgesia did not develop in acid sensing ion channel 3 (ASIC3) knockout mice however did develop in ASIC1 knockout mice. As well, an expansion of the receptive field of the wide dynamic range neurons to include the contralateral paw following innocuous (brush) or noxious (pinch) stimuli was also found when hyperalgesia developed i.e. in all but the ASIC3 knockout mice demonstrating a change in central processing in the spinal cord (Sluka et al., 2003).

Further investigations revealed there was not one uniform mechanism present throughout the entire duration of hyperalgesia. Skyba et al demonstrated that there was no effect with blocking NMDA or non-NMDA receptors before the first acidic saline injection. However, blocking the NMDA receptors before the second acidic saline injection delayed the development of hyperalgesia. There was no effect when the non-NMDA receptors are blocked at this time. Finally, blocking both the NMDA and non-NMDA receptors one week after the development of hyperalgesia decreased the hyperalgesia. Therefore, NMDA receptors may be involved in the development of hyperalgesia and both NMDA and non-NMDA glutamate receptors are involved in the maintenance of the persistent mechanical hyperalgesia (Skyba, King, & Sluka, 2002). Hoeger-Bement and Sluka found levels of CREB and phosphorylated CREB (p-CREB) in the spinal dorsal horn increased at 24 hours after the second injection but not at 1 week. The effectiveness of adenylate cyclase and protein kinase A inhibitors followed similar timeframes with the reversal of mechanical hyperalgesia at 24 hours but not at 1 week (Hoeger-Bement & Sluka, 2003). Skyba et al investigated the concentrations of the excitatory amino acids glutamate and aspartate in L4 and L5 spinal segments finding increased concentrations 90 minutes after the second injection but not after the first injection (Skyba, Lisi, & Sluka, 2005). They further hypothesize that the increased concentrations may sensitize the dorsal horn spinothalamic neurons and contribute to the hyperalgesia. This may in turn increase NMDA and non-NMDA receptor activity. In addition to providing further information about the mechanisms of the acidic saline model, these results highlight that different mechanisms may underlie the onset versus maintenance of hyperalgesia.

Ledeboer et al recently investigated the role of glial cells in maintaining the hyperalgesia in this model. Surprisingly, intrathecal administration of the glial inhibitor fluorocitrate on day 16 did not decrease the mechanical hyperalgesia in this model(Ledeboer et al., 2006). These results were in contrast to the many pain models in which a glial role has been demonstrated as discussed previously and suggest an important fundamental difference in the model. Earlier time points in the acidic saline model, however, have not yet been investigated. Therefore, a role for glial cells in the development of hyperalgesia in this model has not yet been assessed.

Although there have been some investigations into the mechanisms at peripheral and spinal levels, the mechanism for the persistent contralateral mechanical hyperalgesia is not yet clearly understood in this model. There are several reasons why it is unlikely that a peripheral mechanism alone such as muscle tissue damage producing continued primary afferent input is responsible. Minimal tissue damage was observed at the

injection sites, and there were no significant differences on the contralateral mechanical hyperalgesia after the local injection of lidocaine or ipsilateral dorsal rhizotomy.

Sluka et al propose that the hyperalgesia found may be due to changes in the central nervous system (Sluka et al., 2001). They suggest that the required interinjection interval suggests biochemical changes that perhaps sensitize the central nervous system. The contralateral nature of the mechanical hyperalgesia and the increase in receptive fields of the wide dynamic range neurons also suggest a central mechanism. What has not been questioned is whether these central changes occur at simply a spinal level or whether supraspinal sites are involved. These questions can be investigated anatomically as well as biochemically. It is not known if the two injections must occur in the same location for the increased sensitivity to mechanical stimuli to develop. If the first injection was done at different site than the second one and similar widespread hyperalgesia develops, spinal and/or supraspinal mechanisms would be suspected. If the second site was not related spinally to the original site than further investigations into a supraspinal role may be warranted. Additionally, if a different type of manipulation was done that again had no spinal relationship to the original site, a supraspinal mechanism would be suspected. This manipulation may serve as a step in determining a central role in this model of chronic musculoskeletal pain.

Lipopolysaccharide (LPS) Model

Accordingly, a different manipulation has been chosen that has no specific spinal relationship to the gastrocnemius muscle and has, in fact, been shown to induce hyperalgesia through supraspinal mechanisms. Watkins et al have developed a pain model using an intraperitoneal (ip) injection of the bacterial endotoxin lipopolysaccharide (LPS) to cause illness-induced hyperalgesia (Watkins et al., 1994). Tail flick test latencies to radiant heat were decreased as compared to controls for up to two hours post injection. Watkins et al have characterized some of the neurocircuitry involved in the model showing a central influence. Disrupting the afferent projections for the vagus nerve by eliminating the nucleus tractus solitarious in the dorsomedial medulla abolished the lipopolysaccharide-induced hyperalgesia (Watkins et al., 1994) (Wiertelak, Roemer, Maier, & Watkins, 1997). Further, they found that brain site or sites rostral to the mid-mesencephalon were required for hyperalgesia to develop as decerebration ceased the hyperalgesia. There was increased cfos-like immunoreactivity in the nucleus raphe magnus (NRM) (Watkins et al., 1994). Also, bilateral lesions of the descending dorsolateral funiculus blocked the LPS-induced hyperalgesia. The fact that this model can, therefore, be blocked by abolishing ascending, supraspinal and descending mechanisms demonstrates that despite being peripherally administered, the hyperalgesia that develops following ip LPS is clearly a centrally mediated response.

Others have used this model to examine long term effects of LPS treatment including the attenuation of the analgesic effects of opioids (Johnston & Westbrook, 2005). Specifically, 24 hours after LPS injection the ability of morphine to mediate analgesia is severely blunted despite the fact that there is no hyperalgesia in these animals at this time point. This would suggest that there is an alteration in processing even after the

hyperalgesia has resolved. However, pre-treatment with fluorocitrate stops the decrease in morphine analgesia 24 hours after the injection suggesting a role for glial cells in this model as well even after the hyperalgesia has ceased (Johnston et al., 2005). Therefore, the LPS -induced hyperalgesia model is interesting in that it both induces hyperalgesia and appears to activate glia. Importantly, the two processes have very different time courses with the glial activation extending well beyond the period of hyperalgesia. Thus an LPS injection may be used as a means of activating glia cells without interfering with sensory testing done 24hrs later after the hyperalgesia has ceased. These two features raised the question of whether this LPS-induced glial activation could substitute for the first injection in the acidic saline model. If so, this would indicate that glial activation may be mediating the development of hyperalgesia in the acidic saline model even if glia are not involved in the maintenance of the hyperalgesia.

PURPOSE

The overall purpose of this investigation was to further examine a central nervous system role in the acidic saline model of chronic musculoskeletal pain. In Experiment 1, this was done anatomically by varying the location of the first injection. The type of first injection was then altered by introducing the ip LPS injection to see if a different type of first insult still caused the widespread mechanical hyperalgesia. In Experiment 2, the glial inhibitor minocycline was introduced to investigate a role of glia cells in the development of mechanical hyperalgesia in the acidic saline model.

Experiment 1 Effect of Varying Location and Nature of First Injection

Objectives – to determine the effect of varying the anatomical location and nature of the first stimulus (injection) on the development of mechanical hyperalgesia in the acidic saline model. To achieve this, the location of the first injection was varied in the same muscle and to a different muscle. An additional group utilized a different stimulus (ip LPS) reported to induce glial activation to test whether this could be substituted for the first injection.

Experiment 2 Role of Glia in Acidic Saline Model

Objective – to determine if there was a role for glia in the early stages of the acidic saline model in the development of mechanical hyperalgesia

HYPOTHESES AND POSSIBLE OUTCOMES

The null hypothesis for this study was that this model required anatomical specificity i.e. the use of two highly localized injections of acidic saline into the same receptive field of sensory afferents in the lateral gastrocnemius muscle for persistent widespread mechanical hyperalgesia to develop. An alternative hypothesis was that the two injections of acidic saline could occur at different locations in the animal and still result in persistent widespread mechanical hyperalgesia. Furthermore, one ip LPS injection and one injection of acidic saline into a gastrocnemius muscle would also initiate the development of persistent widespread mechanical hyperalgesia.

If the null hypothesis was proven true, the two injections of acidic saline must be given in one muscle for the mechanical hyperalgesia to develop. If the alternate hypothesis was accepted and persistent widespread mechanical hyperalgesia developed with the two injections done in different locations i.e. left and right gastrocnemius muscles, a central role would be suggested in the development of hyperalgesia whether it be spinal or supraspinal or a combination of both. Further, if persistent widespread mechanical hyperalgesia developed with the LPS and acidic saline injections, this would be consistent with supraspinal centers having the potential to supply the necessary priming stimulus to permit a subsequent acidic saline injection to induce hyperalgesia. Aside from implying an anatomical pathway, the ability of ip LPS to substitute for an acidic saline injection also supports the possibility that glial activation may be involved in the development of hyperalgesia in the acidic saline model.

The second null hypothesis was that the administration of the glial inhibitor minocycline before the acidic saline injections would not change the characteristics of the model and widespread mechanical hyperalgesia would still develop. The alternate hypothesis was that the administration of minocycline stops the development of mechanical hyperalgesia. If the null hypothesis was found to be true, it would not appear that the glial cells play a role in the development of hyperalgesia in this acidic saline model. This would be consistent with the results found by Ledeboer et al when they examined the glial function at a later time point in this model(Ledeboer et al., 2006). If the alternate hypothesis was accepted and mechanical hyperalgesia did not develop, there would be strong evidence to support a role for glial cells in the development of widespread mechanical hyperalgesia in this model. As this result would differ from those by Ledeboer et al, it would suggest that the glial cells play a role in the development of hyperalgesia rather than the maintenance of it (Ledeboer et al., 2006).

METHODOLOGY

A. Subjects

Male Sprague-Dawley rats weighing 250-350 grams (Charles River, Quebec) were used in this investigation. All experiments were subject to approval of the University of Manitoba Bannatyne Campus Protocol Management and Review Committee.

B. Muscle Injections

The animals were anesthetized with 2-4% isofluorane and injected with either 100 ul of pH 7.2 sterile saline or 100 ul of pH 4.0 acidic saline (Sluka, 2001). The acidic saline was prepared by adding 0.01 N HCl to saline while continuously testing the pH. The pH was again tested prior to injection to ensure it was within 0.1 pH. The animals were side-lying with the leg to be injected superior with the appropriate knee extended and ankle in neutral at the time of the lateral gastrocnemius injections and side-lying on the leg to be injected with the knee extended and ankle in neutral for the medial gastrocnemius injections.

C. LPS Injections

The animals were anesthetized with 2-4% isofluorane and injected intraperitoneally with 100 ug of bacterial endotoxin lipopolysaccharide (LPS, from *E. coli* serotype 05:B55, Sigma) (Watkins, 1997).

D. Minocycline Injections

The animals were anesthetized with 2-4% isofluorane and injected intraperitoneally with 40 mg/kg minocycline (Sigma)(Raghavendra, Tanga, & DeLeo, 2003). Minocycline is an antibiotic that crosses the blood-brain barrier easily and has been shown to decrease central inflammatory responses and specifically microglial activation (Raghavendra, Tanga, & DeLeo, 2003) (Blandino, Jr., Barnum, & Deak, 2006).

E. Mechanical Hyperalgesia Testing

Von Frey filaments (North Coast Touch Test) were used to test the mechanical withdrawal threshold as the reliability of this method has been previously documented (Gopalkrishnan, 2000). The animals were allowed to acclimate in lucite cubicles on a mesh stand for 30 minutes before testing. Filaments of various bending forces were applied to the plantar surface of both hindpaws in all experimental groups. Filaments with bending forces of 39.2, 58.8, 78.4, 98.0, 147.0, 254.8, and 588.0 mN were used starting with the lowest force. Each filament was applied twice and the mechanical withdrawal threshold was considered the level at which the limb is abruptly lifted for two sequential responses. This threshold was confirmed by re-testing the filaments above and below the withdrawal value. The testing was done before each injection and 24 hours after each injection.

F. Experiment 1 – Effect of Varying Location and Nature of First Injection

Saline

Group 1 - Animals (n=12) were injected with 100 ul of sterile saline in the right lateral gastrocnemius on day 0 and day 5 (see Figure 1). The

above mechanical hyperalgesia testing procedures were followed. The testing was done before each injection and 24 hours after each injection. This group was used as a control for the effects of muscle injections on mechanical withdrawal thresholds as well as changes in withdrawal thresholds over the time course of the experimental protocol (6 days).

Acidic Saline (Groups 2 – 5)

Four groups, each with n=12, were used to assess mechanical hyperalgesia. The first injection varied in location and nature. However, all animals received a second injection of acidic saline in the right lateral gastrocnemius muscle. As such, the acidic saline groups are labelled according to location/nature of the two injections.

Group 2 – Right Lat Gastroc–Right Lat Gastroc

Animals (n=12) were injected with 100 ul of acidic saline in the right lateral gastrocnemius on day 0 and again on day 5 (see Figure 1). The above mechanical hyperalgesia testing procedures were followed. This group was used as a positive control reference group following procedure as previously published i.e. injection 1 and 2 were in right lateral gastrocnemius muscle. All other groups were derivations from this group with the location and type of the first injection varying

Group 3 - Right Med Gastroc–Right Lat Gastroc

Animals (n=12) were injected with 100 ul of acidic saline (pH 4.0) in the right medial gastrocnemius on day 0 and the right lateral gastrocnemius on day 5 (see Figure 1). The above mechanical hyperalgesia testing procedures were followed. This group varied the first stimulus location within the same muscle and, therefore, the same spinal segment.

Group 4 – Left Lat Gastroc–Right Lat Gastroc

Animals (n=12) were injected with 100 ul of acidic saline (pH 4.0) in the left lateral gastrocnemius on day 0 and the right lateral gastrocnemius on day 5 (see Figure 1). The above mechanical hyperalgesia testing procedures were followed. This group varied the first stimulus location to a different muscle although the same spinal segment.

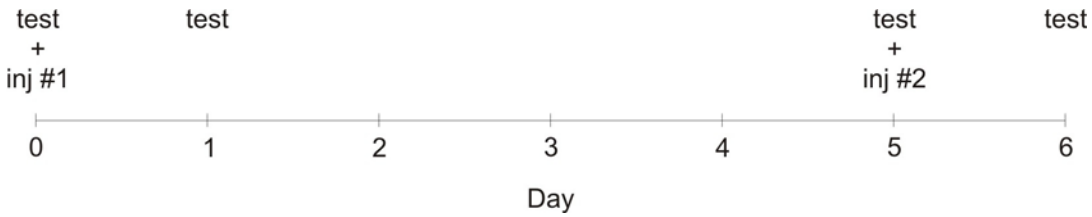

Figure 1. Timeline for two intramuscular saline or acidic saline injections and bilateral hindlimb mechanical withdrawal threshold testing for Expt 1 Group 1 Saline, Expt 1 Group 2 Right Lat Gastroc-Right Lat Gastroc, Group 3 Right Med Gastroc-Right Lat Gastroc and Group 4 Left Lat Gastroc-Right Lat Gastroc.

Group 5 – LPS-Right Lat Gastroc
Intraperitoneal LPS, 100 ug, was injected 24 hours prior to the injection of 100 ul of acidic saline (pH 4.0) in the right lateral gastrocnemius (n=12) (see Figure 2). The above mechanical hyperalgesia testing procedures were followed. This group utilized a first stimulus that had no anatomical relationship to the second stimulus.

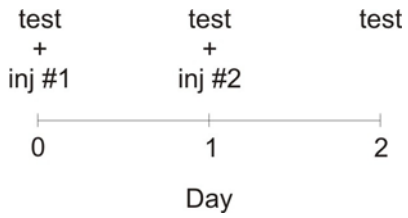

Figure 2. Timeline for intraperitoneal LPS injection (#1), intramuscular acidic saline injection (#2) and bilateral hindlimb mechanical withdrawal testing for Expt 1 Group 5 LPS-Right Lat Gastroc.

G. Experiment 2 - Role of Glia in Acidic Saline Model

Two groups of animals were used varying only the timing of the minocycline injection.

Group 1 – Minocycline pre Injection 1
Animals (n=12) were injected with 100 ul of acidic saline in the right lateral gastrocnemius on day 0 and the right lateral gastrocnemius on day 5. In addition, minocycline, 40 mg/kg, was injected intraperitoneally one hour prior to the first acidic saline injection (see Figure 3). The above mechanical hyperalgesia testing procedures were followed with the baseline test occurring before the minocycline injection.

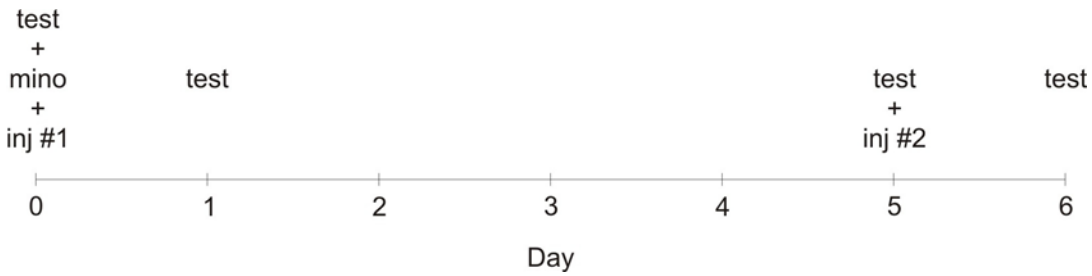

Figure 3. Timeline for two intramuscular acidic saline injections, intraperitoneal minocycline injection and bilateral hindlimb mechanical withdrawal threshold testing for Expt 2 Group1 Minocycline pre Injection 1.

Group 2 – Minocycline pre Injection 2

Animals (n=12) were injected with 100 ul of acidic saline in the right lateral gastrocnemius on day 0 and the right lateral gastrocnemius on day 5. In addition, minocycline, 40 kg/mg, was injected intraperitoneally one hour prior to the second acidic saline injection (see Figure 4). The above mechanical hyperalgesia testing procedures were followed with the baseline test occurring before the minocycline injection.

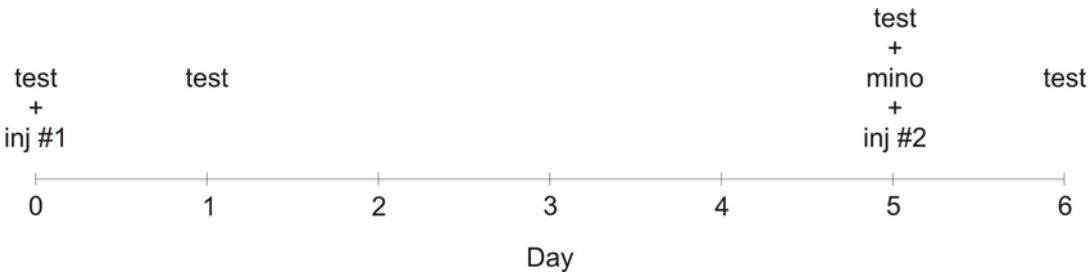

Figure 4. Timeline for two intramuscular acidic saline injections, intraperitoneal minocycline injection and bilateral hindlimb mechanical withdrawal threshold testing for Expt 2 Group 2 Minocycline pre Injection 2.

H. Statistical Analysis

Owing to logistical considerations, not all treatment groups could be included in each experimental block. Further, in recognition of the overall need to reduce the number of experimental animals used in research, each experimental block did not include both saline and acidic saline controls. However, each experimental block included treatment groups that developed hyperalgesia and thereby provided a positive control within each block. This approach ensured that hyperalgesia, if present, could be detected within each experimental block.

Consistent with other laboratories, the response rate was not 100% (Nielsen, 2004). Under observation, it was quite apparent when a positive response occurred in an animal and the mechanical withdrawal threshold lowered. For statistical purposes, however, a responder was defined using two criteria. First, the mechanical withdrawal threshold must have decreased to 98 mN or less 24 hours after the second injection. Second, the mechanical withdrawal threshold must have decreased at least two filaments before the second injection to 24 hours after the second injection. This definition was based on several observations. No saline injected animal presented with a threshold below 147 mN at baseline before the second injection. Further, the 95% confidence limits for the baseline before the second injection timepoints were 232.5 mN to 463.3 mN showing that 98 mN (the bending force of the subsequent lower filament) or less was well outside of these limits. Also, no saline injected animal presented with a threshold below 147 mN 24 hours after the second injection. Further, the 95% confidence limits for 24 hours after the second injection timepoints were 290.9 mN to 506.1 mN showing that 98 mN or less was, again, well outside of these limits. Therefore, neither pre nor post injection 2 was

the withdrawal threshold in saline treated animals below 147 mN allowing the conclusion that these two criteria do not allow for values occurring spontaneously over time or as a result of the muscle injection itself. Also, no saline injected animal showed downward movement of two filaments between baseline before second injection and 24 hours post second injection. These two factors provided both an absolute (98 mN or less) criterion and a relative criterion (decrease of at least two filaments) to create a stringent definition of hyperalgesia (responder). In groups where animals developed hyperalgesia, statistical analysis was limited to those animals demonstrating a hyperalgesic response.

Mechanical withdrawal thresholds can obviously vary across a continuous scale. However, the assessment tool used, although clinically relevant, is both discrete and non-linear i.e. 39.2, 58.8, 78.4, 98.0, 147.0, 254.8, and 588.0 mN. Accordingly, a logarithmic transformation of the filament bending forces was performed to create a linear scale. Doing so creates statistical additivity, a requirement for parametric statistical analysis (Zar, 1987) and gives greater meaning to the use of a decrease of at least 2 filament levels as a criterion for defining the development of hyperalgesia. This is consistent with other publications using logarithmic transformations to allow parametric statistical analysis (Spataro, 2004). To test the main effects of side and time, the parametric two-way ANOVA test was used. Posthoc testing was done using Tukey's test. $P<0.05$ was considered to be statistically significant. Statistica version 5.1 from Statsoft, Inc was used for the analysis.

RESULTS

A. Experiment 1 – Effect of Varying Location and Nature of First Injection

Group 1 - Saline Group

As expected, none of the animals receiving saline injections developed hyperalgesia (0/12; 0% responders, Table 1). There were no statistically significant main effects for side ($F_{1,5}$=3.544, p=0.118) or time ($F_{3,15}$=2.806, p=0.075) nor was the side x time interaction significant ($F_{3,15}$=0.243, p=0.865). The saline group demonstrated a stable response throughout the testing timeframes with no statistically significant changes after the first (ipsilateral: p=0.053; contralateral: p=0.369) or second injections (ipsilateral: p=0.999; contralateral: p=0.999). There was also no significant difference between the baselines before the first and second injections (ipsilateral: p= 0.369; contralateral: p= 0.639). Thus, there is no tendency towards hyperalgesia exhibited at any timepoint in saline treated animals.

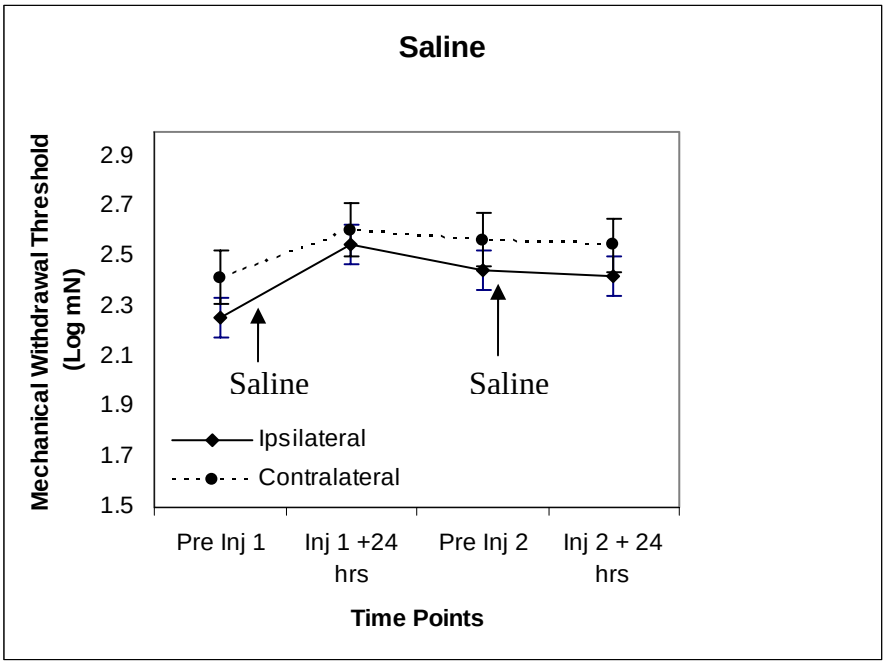

Figure 5. Group 1 Saline: Hindlimb ipsilateral and contralateral mechanical withdrawal thresholds of rats receiving intramuscular injections of saline on Day 0 and Day 5. No change in withdrawal thresholds was observed following the second injection.

Group 2 – Right Lat Gastroc–Right Lat Gastroc

Six of the 12 animals (50%) receiving both injections of acidic saline in the right lateral gastrocnemius muscle developed a clear mechanical hyperalgesia (Table 1). Neither the main effect of side ($F_{1,5}$=2.851, p=0.152) nor the side x time interaction ($F_{3,15}$=2.999, p=0.064) were statistically significant. However, the main effect of time was highly significant ($F_{3,15}$=11.291, p<0.001). Similar to

saline treated animals, baseline withdrawal thresholds immediately prior to each injection were not different (ipsilateral: p= 0.085; contralateral: p= 0.974) nor did withdrawal responses change after the 1st injection (ipsilateral: p=1.000; contralateral: p= 0.795). In contrast to the saline group, the withdrawal threshold dropped significantly after the 2nd injection of acidic saline (ipsilateral: p< 0.001; contralateral: p<0.001). This data indicates the bilateral development of hyperalgesia.

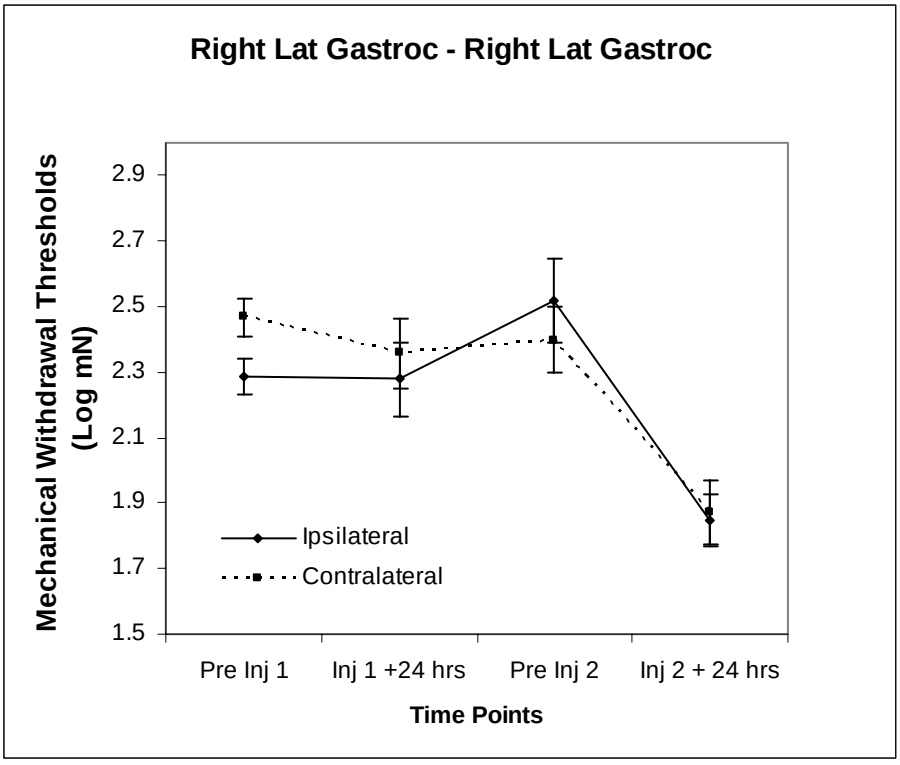

Figure 6. Group 2 Right Lat Gastroc–Right Lat Gastroc: Hindlimb ipsilateral and contralateral mechanical withdrawal thresholds of rats receiving intramuscular injections of acidic saline on Day 0 and Day 5. Significant decreases in bilateral withdrawal thresholds were observed following the second injection indicating the development of bilateral hyperalgesia.

Group 3 - Right Med Gastroc–Right Lat Gastroc

Eight of the 12 animals (67%) receiving the first injection of acidic saline in the right medial gastrocnemius muscle and the second injection of acidic saline in the right lateral gastrocnemius muscle developed a clear mechanical hyperalgesia (Table 1). A significant effect of side ($F_{1,7}$=7.264, p= 0.031) and time ($F_{3,21}$=29.239, p<0.001) was present in the two way ANOVA. There was no significant side by time interaction indicating that any side-to-side differences were consistent throughout the experiment ($F_{3,21}$=1.716, p=0.194). There was a significant difference between the baselines before the first and second injections (ipsilateral: p= 0.021; contralateral: p< 0.001) with the baseline before the second injection slightly higher. There was no significant difference between pre- and

post-injection withdrawal thresholds for the first injection (ipsilateral: p=0.809; contralateral: p=0.809). Importantly, there were significant differences between baseline and 24 hours after the second injection (ipsilateral: p< 0.001; contralateral: p< 0.001) indicating bilateral hyperalgesia.

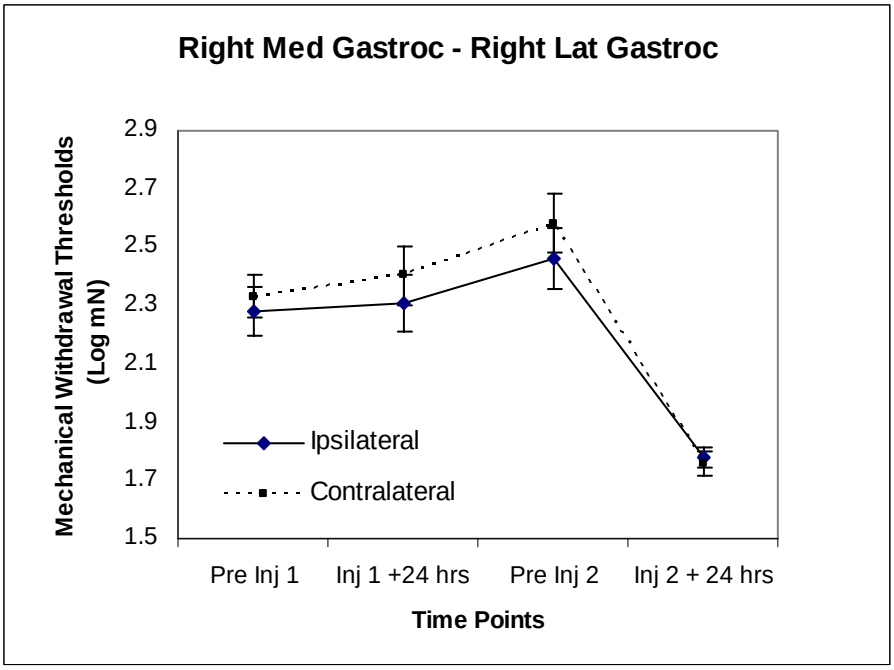

Figure 7. Group 3 Right Med Gastroc–Right Lat Gastroc: Hindlimb ipsilateral and contralateral mechanical withdrawal thresholds of rats receiving intramuscular injections of acidic saline on Day 0 and Day 5. Significant decreases in bilateral withdrawal thresholds were observed following the second injection indicating the development of bilateral hyperalgesia.

Group 4 – Left Lat Gastroc–Right Lat Gastroc

Six of the 12 animals (50%) receiving the first injection of acidic saline in the left lateral gastrocnemius muscle and the second injection of acidic saline in the right lateral gastrocnemius muscle developed a clear mechanical hyperalgesia (Table 1). Significant effects were found for side ($F_{1,5}$=16.331, p= 0.001) and time ($F_{3,15}$=55.157, p<0.001). A significant side by time interaction was not found ($F_{3,15}$=1.539, p=0.245). There were no significant differences between the baselines before the first or second injections (ipsilateral: p=0.143; contralateral: p=1.000). There was no significant difference between withdrawal thresholds before the first injection and 24 hours after the first injection (ipsilateral: p=0.626; contralateral: p=0.950). There was however significant differences between baseline and 24 hours after the second injection (ipsilateral: p< 0.001; contralateral: p< 0.001) again indicating bilateral hyperalgesia.

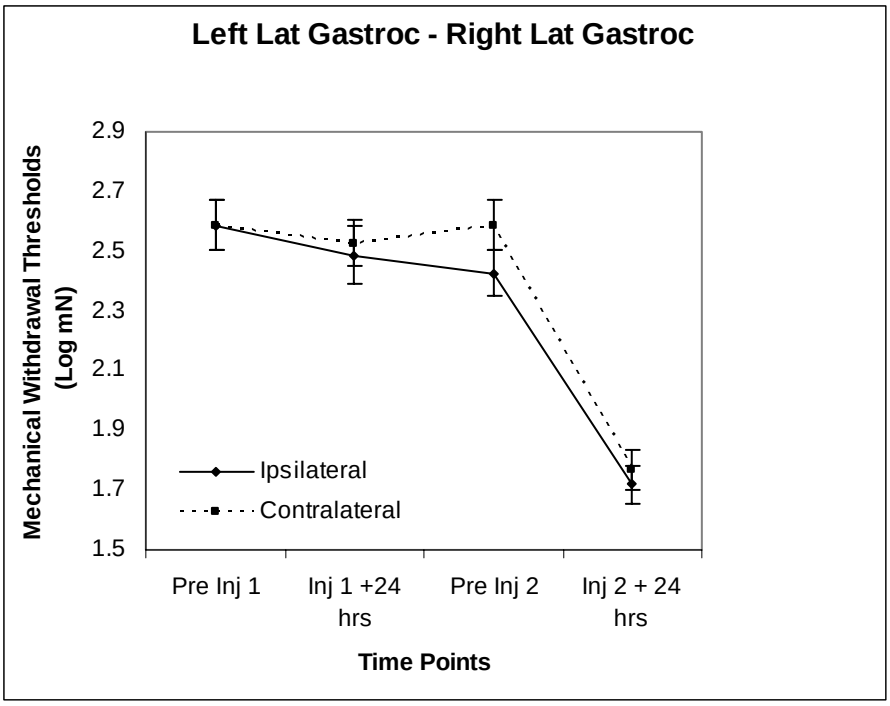

Figure 8. Group 4 Left Lat Gastroc–Right Lat Gastroc: Hindlimb ipsilateral and contralateral mechanical withdrawal thresholds of rats receiving intramuscular injections of acidic saline on Day 0 and Day 5. Significant decreases in bilateral withdrawal thresholds were observed following the second injection indicating the development of bilateral hyperalgesia.

Group 5 – LPS-Right Lat Gastroc

Two of the 12 animals (17%) receiving ip LPS as the first injection and a second injection of acidic saline in the right lateral gastrocnemius muscle developed a clear mechanical hyperalgesia (Table 1). As only two animals demonstrated a hyperalgesic response, it was not possible to use the ANOVA procedure. However, these 2 animals had a similar response pattern as that seen in animals that developed hyperalgsia in other groups. There was little difference in the values until twenty-four hours after the second injection when the values decreased bilaterally, demonstrating a hyperalgesic response.

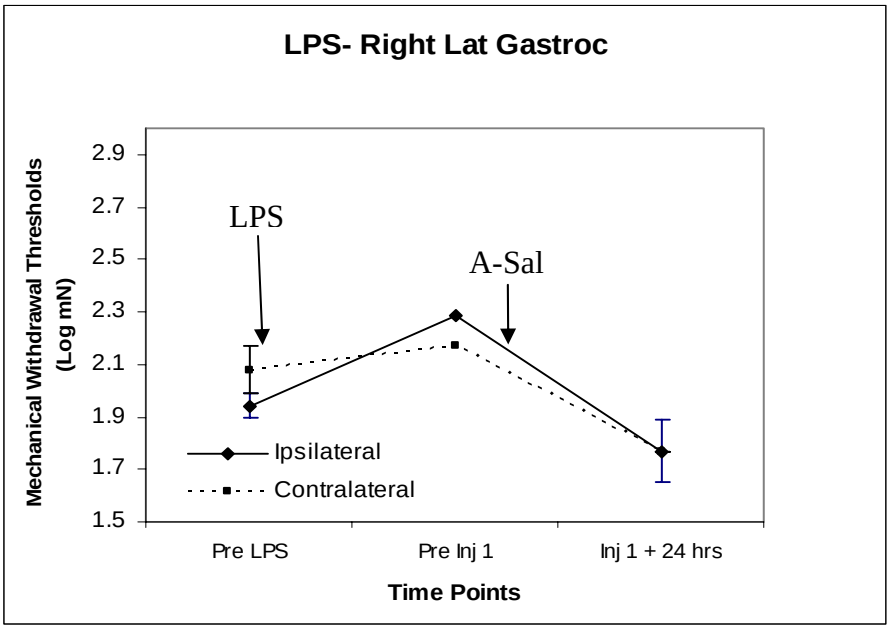

Figure 9. Group 5 LPS-Right Lat Gastroc: Hindlimb ipsilateral and contralateral mechanical withdrawal thresholds of rats receiving an intraperitoneal injection of LPS 24 hours before one intramuscular injection of acidic saline. Decreases in bilateral withdrawal thresholds were observed following the acidic saline injection indicating the development of bilateral hyperalgesia.

Table 1 Experiment 1 Response Rates

Group	# Responders	Percentage Response (%)
1. Saline	**0/12**	**0**
2. Right Lat Gastroc-Right Lat Gastroc	**6/12**	**50**
3. Right Med Gastroc-Right Lat Gastroc	**8/12**	**67**
4. Left Lat Gastroc-Right Lat Gastroc	**6/12**	**50**
5. LPS-Right Lat Gastroc	**2/12**	**17**

B. Experiment 2 – Role of Glia in Acidic Saline Model

Group 1 – Minocycline pre Injection 1

None of the 12 animals (0%) receiving minocycline i.p. before the first injection of acidic saline in the right lateral gastrocnemius muscle developed a clear mechanical hyperalgesia (Table 2). No significant effects were found for side ($F_{1,5}$=0.476, p=0.521) or time ($F_{3,15}$=2.498, p=0.099) in the two way ANOVA. The time effect was approaching significance due to a slight increase after injection 1 showing absolutely no change or possibility of hyperalgesia. There was also no significant side x time interaction ($F_{3,15}$=1.116, p=0.374). There were no significant differences between the baselines before the first or second injections (ipsilateral: p=1.000; contralateral: p=0.740). There was no significant

difference between withdrawal thresholds before the first injection and 24 hours after the first injection (ipsilateral: p= 0.603 and contralateral: p= 0.251). Most importantly, no significant differences were found between withdrawal thresholds at baseline and 24 hours after the second injection (ipsilateral: p=0.962; contralateral: p=0.999) indicating hyperalgesia did not develop in this group.

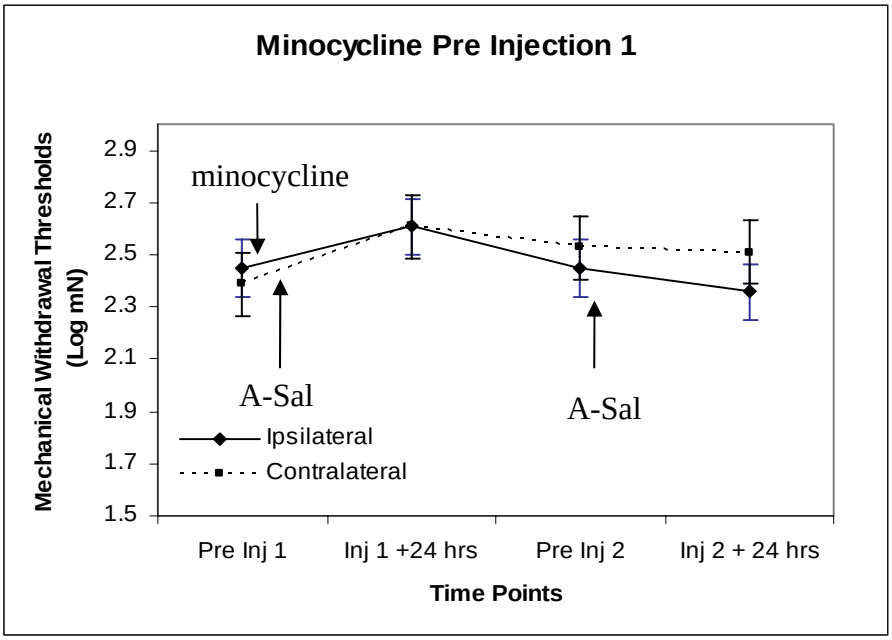

Figure 10. Group 1 Minocycline pre Injection 1: Hindlimb ipsilateral and contralateral mechanical withdrawal thresholds of rats receiving injections of acidic saline on Days 1 and 5 with a minocycline injection before the first acidic saline injection. No change in withdrawal thresholds was observed following the second injection demonstrating hyperalgesia did not develop.

Group 2 – Minocycline pre Injection 2

When the minocycline was administered before the second injection, six out of twelve (50%) animals developed hyperalgesia (Table 2). Significant effects were found for time ($F_{3,6}$=72.585, p<0.001). No significant effects of side ($F_{1,2}$=11.682, p=0.076) or side by time interaction ($F_{3,6}$=2.350, p=0.172) were found. There were no significant differences between the baselines before the first and second injections (ipsilateral: p=1.000; contralateral: p=0.512). There was no significant difference between withdrawal thresholds before the first injection and 24 hours after the first injection (ipsilateral: p=0.753; contralateral: p= 0.950). Interestingly, there was a significant difference between baseline and 24 hours after the second injection (ipsilateral: p< 0.001; contralateral: p< 0.001) clearly demonstrating the development of bilateral hyperalgesia in this group.

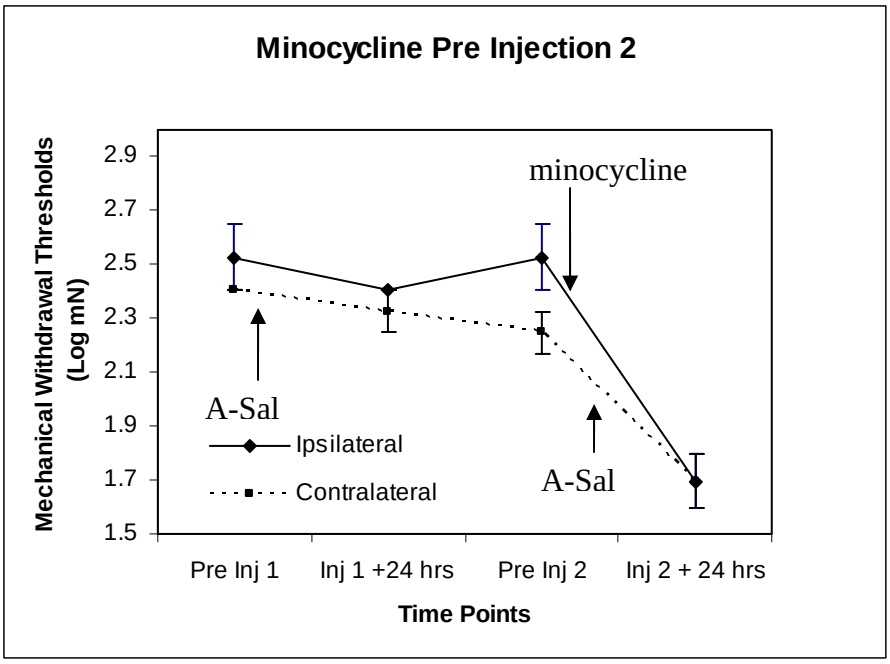

Figure 11. Group 2 Minocycline pre Injection 2: Hindlimb ipsilateral and contralateral mechanical withdrawal thresholds of rats receiving injections of acidic saline on Days 1 and 5 with minocycline before the second acidic saline injection. Significant decreases in bilateral withdrawal thresholds were observed 24 hours following the second acidic saline injection indicating the development of bilateral hyperalgesia.

Table 2 Experiment 2 Response Rates

Group	# Responders	Percentage Response (%)
1. Minocycline pre Inj 1	0/12	0
2. Minocycline pre Inj 2	6/12	50

DISCUSSION

Anatomical Specificity of Acidic Saline Model

This investigation clearly demonstrates that the two injections of acidic saline do not have to be in the same location for mechanical hyperalgesia to develop. Furthermore, the first injection of acidic saline can be replaced with a different type of stimulus and still cause the development of hyperalgesia.

In previous publications of the acidic saline model, both acidic saline injections were given in the right lateral gastrocnemius muscle perhaps in an attempt to stimulate the same local tissue, sensory afferent pool, dorsal horn neurons and supraspinal pathways.

When the first acidic saline injection was relocated to the medial head of the right gastrocnemius muscle, mechanical hyperalgesia developed bilaterally. Obviously, different local tissue and sensory afferent pools are being targeted with the two injections although similar dorsal horn cells and supraspinal pathways may be accessed. This suggests that peripheral mechanisms localized to the muscle injection site can not alone account for the development of hyperalgesia. It does not appear that any type of priming or sensitizing event in the local tissue is required for hyperalgesia to develop as the two injections in different locations still resulted in hyperalgesia.

When the first acidic saline injection was moved to the lateral head of the left gastrocnemius, mechanical hyperalgesia again developed bilaterally providing further evidence that peripheral mechanisms cannot be solely responsible for the hyperalgesia. The first injection was again clearly done in different local tissue and different sensory afferent pools. Further, it is likely that different areas of the dorsal horns were stimulated by the contralateral muscle injection. Although there is some evidence that central afferent projections may terminate in the contralateral dorsal horn these projections are relatively rare (Verkhratsky et al., 2007; Novikov, 2001; Sugiura, Terui, & Hosoya, 1989; Smith, 1983). Lastly, no responses were recorded in wide dynamic range neurons when the contralateral limb was subjected to brush or pinch stimuli (Sluka et al., 2003). Thus, the relatively minor nature of the contralateral projections reported to date make it unlikely that this can account for the ability of acidic saline injection in the contralateral gastrocnemius to induce bilateral hyperalgesia.

To extend this series of experiments, the first injection was moved to a site not related anatomically to the first i.e. intraperitoneal vs. right lateral gastrocnemius muscle and the nature of the stimulus was also changed i.e. LPS rather than acidic saline. It is interesting that mechanical hyperalgesia still developed raising the question of the mechanism that can be initiated by two different noxious stimuli. It would appear that the two injections did not access the same local tissue, primary afferent pool or dorsal horn neurons. Collectively, these data indicate that the development of hyperalgesia in the acidic saline model may utilize a similar supraspinal mechanism as reported by Watkins et al (Watkins et al., 1994)

Sluka et al hypothesized the hyperalgesia may be due to changes in the central nervous system (Sluka et al., 2001). The required interinjection interval of 2 -5 days suggests the central nervous system may have become sensitized by time-limited biochemical changes. A central mechanism is also suggested by the bilateral nature of the mechanical hyperalgesia and the increase in receptive fields of wide dynamic range neurons to include the contralateral limb (Sluka et al., 2003). Skyba et al hypothesized that the increased concentrations of glutamate and aspartate 90 minutes after the second acidic saline injection suggest sensitization of the dorsal horn spinothalamic neurons and may contribute to increased NMDA and non-NMDA receptor activity (Skyba, Lisi, & Sluka, 2005). Determining the role of central mechanisms in the acidic saline model of chronic pain would provide further insight into the development of chronic pain and hopefully encourage improvements in the treatment and prevention of chronic pain.

Response Rates

Consistent with other laboratories, not all of the experimental animals developed hyperalgesia. Nielsen et al reported a 72% responder rate (Nielsen, 2004) and in discussion with Dr. Sluka, an approximately 80% responder rate was reported.

In our investigation, response rates varied across experimental groups. As expected, the saline control group had 0/12 (0%) responders. The acidic saline group with both injections in the right lateral gastrocnemius muscle had 6/12 (50%) responders.

The group with injection 1 in the right medial gastrocnemius and injection 2 in the right lateral gastrocnemius had an 8/12 (67%) response rate. The group with injection 1 in the left lateral gastrocnemius and injection 2 in the right lateral gastrocnemius had a response rate of 6/12 (50%). These results clearly show that the alternate locations for the first injections still allow for the development of mechanical hyperalgesia.

The group with an intraperitoneal LPS injection as injection 1 and the acidic saline in the right lateral gastrocnemius muscle as injection 2 had a response rate of 2/12 (17%). This rate is obviously lower than the published 70% but as it still had clear responders present does demonstrate that this type of injection and stimulus can cause the development of hyperalgesia. This group was not appropriate to investigate the role of glia using the minocycline due to the low response rate. This group would, however, be valuable to study variables which may increase the response rate. Indeed, further investigation into why some animals develop hyperalgesia and others do not would be very informative and relevant clinically. There is currently no literature on this aspect likely due to the fact that mechanisms specific to the acidic saline model of hyperalgesia have yet to be identified. Perhaps a first step would be to record muscle afferent activity to examine if consistent input occurred from the peripheral injections. If little variability was found, specific mechanisms for hyperalgesia must continue to be sought in order to provide baselines for comparison between responders and nonresponders. Uncovering these mechanisms may have great clinical significance with potential to reveal critical mechanisms underlying chronic muscle pain.

Hyperalgesia vs. Allodynia

The International Association for the Study of Pain (IASP) defines hyperalgesia as an increased response to a stimulus which is normally painful. This represents an increased perception to pain within the original modality. The IASP defines allodynia as pain due to as stimulus which does not normally provoke pain and emphasizes that the original modality is not painful. Further, A-beta fibres have been implicated in allodynia through central alterations (Koltzenburg et al., 1992; Torebjork, Lundberg, & LaMotte, 1992; Koltzenburg et al., 1994; Klede, Handwerker, & Schmelz, 2003). In publications from laboratories using the acidic saline model, both the terms hyperalgesia and allodynia are used to describe the phenomenon demonstrated by lowered mechanical withdrawal thresholds (Gandhi, 2004; Ledeboer et al., 2006; Skyba et al., 2002; Sluka et al., 2001). This lack of consistency of terms may be due to the difficulty in determining which actual fibres are sensing the pressure of the Von Frey filaments at the level of withdrawal. One laboratory used the term mechanical hypersensitivity perhaps due to this uncertainty (Nielsen, 2004). For the purpose of this study, the term hyperalgesia was used in order to be consistent with terminology used by the majority of those working with this model.

Role for Glia in Hyperalgesia

The fact that minocycline administered before the first acidic saline injection blocks the hyperalgesic response supports a role for glia in the development hyperalgesia. This is consistent with other models such as intraplantar injection of formalin (Fu et al., 1999; Sweitzer et al., 1999; Watkins et al., 1997), ligation of spinal nerve and nerve roots (Hashizume et al., 2000; Winkelstein et al., 2001) and complete Freund's adjuvant induced arthritis (Raghavendra et al., 2004). A role for glia has also been demonstrated in the LPS model. When examining the long term effects of LPS, specifically the attenuation of the analgesic effects of opioids, pre-treatment with the glial inhibitor fluorocitrate prevented the decrease in morphine analgesia 24 hours after the LPS injection suggesting a role for glial cells in this model as well even after the hyperalgesia has ceased (Johnston et al., 2005).

In contrast, others have found no role for glial cells in the acidic saline model once the hyperalgesia has been established. This was the case when intrathecal delivery of the glial inhibitor, fluorocitrate, failed to reverse the mechanical hyperalgesia (Ledeboer et al., 2006). However, our investigation was the first to examine the role of glia at an earlier time point in the acidic saline model. We further narrowed down the critical timeframe for glial involvement by administering minocycline separately either before the first or second acidic saline injection. The fact that minocycline before the first acidic saline injection stopped the development of hyperalgesia but did not prevent the hyperalgesia when administered before the second acidic saline injection suggests a role for glia in the early stages of the model. Supporting this suggestion are studies not only showing a role for glia in the initiation of hyperalgesia but suggesting that microglia may be more important for the initiation of hyperalgesia with astrocytes being more important for maintenance of hyperalgesia (Colburn, Rickman, & DeLeo, 1999; Raghavendra, Tanga, & DeLeo, 2003). The fact that glia appear to have a role in the early stages but not the later stages in the acidic saline model is consistent with several other findings that are time dependent. Blocking NMDA but not non-NMDA receptors delays the onset of

hyperalgesia however both NMDA and non-NMDA receptors are involved in the maintenance of the hyperalgesia (Skyba et al., 2002). P-CREB and enhanced CREB expression was present in the spinal dorsal horn at 24 hours after the second injection but not after 1 week (Hoeger-Bement et al., 2003). Adenylate cyclase and protein kinase A inhibitors also reverse hyperalgesia at 24 hours after the second injection but not after 1 week (Hoeger-Bement et al., 2003). These results suggest there may be differences in mechanisms of initiating versus maintaining hyperalgesia.

The precise mechanism through which glial cells contribute to hyperalgesia is not known. Spataro et al hypothesize that spinal gap junctions between glial cells may be involved in the spread of hyperalgesia as using the gap junction decoupler carbenoxolone stops the development of mirror pain while not affecting the ipsilateral mechanical allodynia (Spataro et al., 2004). Other laboratories have investigated the role of mediators released by glial cells. Examples include nitric oxide(Minghetti & Levi, 1998), cytokines (Hanisch, 2002), and amino acids (Araque, Carmignoto, & Haydon, 2001). With increasing evidence for the role of glia in hyperalgesia, identifying this mechanism may be an important step to improving the pharmaceutical and clinical management of hyperalgesia.

Fibromyalgia

The acidic saline model of chronic pain has been suggested as a possible model of fibromyalgia (Sluka et al., 2001). There are several parallels with the most obvious similarities being the widespread tenderness found in fibromyalgia and the bilateral hyperalgesia in acidic saline model and the chronicity of both (Vierck, 2006)(Sluka et al., 2001). Similarly, there is no obvious ongoing peripheral input in either situation (Sluka et al., 2001) (Vierck, 2006). Both people with fibromyalgia and animals in the acidic saline model have been shown to respond favorably to aerobic exercise (Vierck, 2006) (Hoeger-Bement et al, 2005). Interestingly, a role for acid sensing ion channels 3 (ASIC3) has been clearly implicated in the acidic saline model and has also been suggested as a pharmaceutical target in the treatment of fibromyalgia (Sluka et al., 2003)(Vierck, 2006). Further study of fibromyalgia is required; however, the acidic saline model allows the development of potential mechanistic hypotheses that can eventually be applied to the human condition.

CONCLUSION

In spite of a large body of research dedicated to the study of pain, there are few models that are chronic and muscle-based. The acidic saline model provides a unique model of pain that is chronic in nature, does not involve muscle damage, does not depend on continuing primary afferent input yet causes persistent widespread mechanical hyperalgesia. This model has similarities with many human chronic pain conditions such as fibromyalgia. The model is also unique in that two insults are required before the widespread mechanical hyperalgesia develops raising the question of what time-limited mechanism is occurring and perhaps sensitizing the animal to a second insult. Peripheral mechanisms have historically undergone more research perhaps leading to our better understanding and treatment of acute rather than chronic pain. Hopefully by gaining a better understanding of central mechanisms, our treatment of chronic pain will improve. Identifying the mechanisms through which the hyperalgesia develops in this model will hopefully allow insight into mechanisms of human chronic pain. Targeting specific hyperalgesia-related sites through physical or pharmacological treatments will only be possible after identifying the mechanisms that contribute to the phenomenon of hyperalgesia. It will be interesting to see how traditional assessments and therapies change with better understanding of these mechanisms. What is clear is that traditional therapies based on acute pain models do not work for chronic pain. It is hopeful that re-examining chronic pain from a hyperalgesia perspective will guide medical professionals in assessment and treatment. This approach may help predict which treatments will be most effective for a given client, at what time the intervention is appropriate, who is at risk for chronic pain and assist in the appropriate allocation of health funds. And most importantly, a better understanding of pain can help improve the quality of life for the millions of people affected by chronic pain.

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