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Purpose: Many elderly experience inadequate postoperative pain relief resulting in increased morbidity and mortality. Several experimental models of postoperative pain have been developed but none were adapted to study the effects of aging on the postoperative pain. Review of literature explored current knowledge of postoperative pain models and identified several models suitable for aging studies. A unique model of postoperative pain, the dorsal hairy skin incision model, was modified and adopted for aged rats. Using this model, we tested two hypotheses: a) aged rats will exhibit similar intensity but longer duration of postincision hyperalgesia compared to young rats and b) spinal cord NR1 expression will increase in response to nociceptive stimulation and that age-related differences in magnitude of NR1 expression will be evident.

Methods: In study I, young (5-7 months old) and aged (22-23 months old) male Fischer 344 rats were exposed to nociceptive testing with von Frey filaments and the *cutaneous trunci* muscle reflex was measured. For each stimulation, a graded response of 0, 0.5, or 1, for no reflex, a small reflex, or vigorous reflex, respectively was recorded. After baseline testing, a 2 cm incision was made through the dorsal skin followed by skin closure and recovery. Subsequently, rats were tested at 3 hours, 6 hours, and on postoperative days (POD) 1, 3, 6, 10, and 14. In study II, young (4-6 months old) and aged (19-21 months old) male Fischer 344 rats were subjected to three sessions of

mechanical nociceptive stimulus. After testing, spinal cords were harvested for western blot analysis of NR1 expression.

Results: In study I, aged rats had greater baseline graded responses to nociceptive stimuli. After incision, young rats developed primary allodynia lasting until POD 3 and primary hyperalgesia until POD 8. Aged rats did not develop allodynia or primary hyperalgesia. Neither group developed secondary hyperalgesia. Aged rats demonstrated greater sensitivity to baseline nociceptive testing and greater maximal graded responses to repetitive testing sessions. In young rats, nociceptive stimulation resulted in a significant increase in NR1 expression. Increased NR1 expression in young tested rats positively correlated with an increase in graded response for one of 18 session/region/force categories tested. There was no increase in spinal cord NR1 expression in aged rats in response to nociceptive stimulation. Low NR1 expression in aged tested rats negatively correlated with an increase in graded response for 9 of 18 session/region/force categories tested.

Conclusions: An experimental rat model to study effects of age on postoperative pain is presented. Age has a profound impact on the pre- and postoperative periods. Aged rats differ significantly from young rats in sensitivity and maximal graded response to acute incisional pain. Young rats exposed to mechanical punctate nociceptive stimuli experienced increased NR1 expression which positively correlated with an increase in graded response. In contrast, aged rats with decreased NR1 expression negatively correlated with an increased graded response. Lower sensitivity and maximal graded responses in the young rats reflect an intact endogenous modulatory pain pathway.

Greater sensitivity and maximal graded responses in the aged rats reflect impairment of descending modulatory pain pathways.

AGING CONFERS AN INCREASE IN SENSITIVITY AND SENSITIZATION TO PAIN AND RESULTS IN SHIFTS OF SPINAL NR1 EXPRESSION

DISSERTATION

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My parents, family, friends, and Art for their continuous love and support during this journey

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CHAPTER I

INTRODUCTION

The following document addresses the issue of inadequately treated postoperative pain in the elderly patient. While numerous studies have examined differences between adults and the elderly in response to surgical procedures, in particular, pain, basic mechanistic differences between the two groups has yet to be elucidated.

Chapter II reviews neurophysical changes with aging and preclinical rodent models of postoperative pain. Adaptation of the dorsal skin model to the aged rat is introduced. In Chapter III the postoperative pain model is adopted and modified for use in the aged rat. Results of these studies are presented. Interestingly, aged rats failed to demonstrate significant hyperalgesia in response to a dorsal skin incision. However, differences in baseline sensitivity and sensitization to repetitive von Frey filament testing were noted. The studies in Chapter IV examined differences in spinal cord expression of the N-methyl-D-aspartate receptor NR1 subunit in two age groups of Fischer 344 rats. Spinal cord NR1 expression in naïve rats was compared to rats subjected to von Frey filament testing. Aged rats demonstrate greater sensitivity and sensitization to repeated nociceptive stimulation than young rats which may be related to degeneration of descending modulatory pain pathways.

Outcomes of these studies provide direction in further research aimed at ultimately improving therapeutic modalities for postoperative pain in the elderly population.

AGING AND ACUTE POSTOPERATIVE PAIN MODELS

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CHAPTER II

AGING AND ACUTE POSTOPERATIVE PAIN MODELS

Abstract

People are living longer, and consequently, more elderly are undergoing surgical procedures. In the past, these individuals would have been excluded from surgical consideration based solely on chronological age. However, postoperative pain management in the elderly is grossly inadequate with almost half of cognitively intact patients reporting moderate to severe pain. Postoperative pain therapy is complicated by the often frail elderly who may have co-morbidities and often take many medications. Age-related changes in the peripheral and central nervous systems alter pain processing and perception. The deterioration of descending inhibitory pathways allows greater afferent input into the central nervous system. Despite these alterations, elderly report similar pain intensity compared to adults but exhibit greater stoicism and reluctance to rate noxious stimuli as unpleasant. Recently, several experimental rodent models have been developed to study postoperative pain but none of these models have been used to study postoperative pain in aged rats. These models can be adapted to study the effect of aging on various aspects of postsurgical pain. It is important to discern the differences in postoperative pain between young and aged rats. Better understanding of the effects of advanced age on postoperative pain will allow us to develop more effective pain management strategies and subsequently, to reduce morbidity and mortality in the elderly after surgery.

Introduction

The population of individuals over the age of 65 is growing rapidly in the US. In 2006, 37 million Americans were over the age of 65, representing over 12% of the population (Robinson 2008). By 2030, the numbers are projected to increase dramatically to 71.5 million individuals over 65 years and 9.6 million over the age of 85 (Robinson 2008).

The elderly, especially those over 85 years of age, are frail due to age-related co-morbidities (Taylor et al. 2005) and general decline in basic organ function with age (Buchner and Wagner 1992). Physical frailty is defined as "a state of reduced physiological reserve associated with increased susceptibility to disability" (Buchner and Wagner 1992). Major components of frailty include reduced physiologic capacity in neurologic control, mechanical performance, and energy metabolism. Surgery and postoperative pain can result in deleterious physiological changes and lead to additional co-morbidities in the elderly and thus, bring about unique challenges for healthcare providers. Surgical incision and manipulation of tissue activates nociceptors on primary afferent fibers and transmission of pain to the central nervous system (CNS) leading to activation of autonomic and somatic reflexes. This complex response involves neuroendocrine, respiratory, cardiovascular, gastrointestinal, CNS, renal, immune system, and coagulation changes (Aubrun 2005; Richardson and Bresland 1998). Preventing activation of autonomic responses through control of nociception is imperative in the frail elderly to prevent or reduce the deleterious effects that accompany nociception.

Healthcare and therapeutic outcomes in care of the elderly may be further complicated by cognitive impairment. The prevalence of cognitive decline increases with advancing age (Robinson 2008). Moderate to severe dementia afflicts 32% of the individuals age 85 and older

which is six times higher than the 5% of individuals age 65-69 (Robinson 2008). Impaired communication further complicates care of the cognitively impaired elderly.

The elderly often receive inadequate postoperative pain relief (Karani and Meier 2004; Lynch et al. 1997; Maxam-Moore, Wilkie, and Woods 1994; Morrison and Siu 2000) and insufficient care in alleviating chronic pain conditions (Bernabei et al. 1998). In a study on pain in patients with hip fracture, Morrison and Siu (2000) discovered that 44% of cognitively intact patients reported severe to very severe pain preoperatively and 42% reported similar pain postoperatively . Patients with advanced dementia received only one-third the amount of opioid analgesics that cognitively intact (and in severe pain) patients received, implying that the majority of demented patients were in severe pain postoperatively. Primary reasons for inadequate pain relief include lack of proper pain assessment and concerns about potential risks of polypharmacy in the elderly (Hitchcock, Ferrell, and McCaffery 1994; Von Roenn et al. 1993). Elderly frequently have co-morbidities that can complicate anesthesia, surgical procedure, and recovery (Taylor et al. 2005). Treatment of postsurgical pain with opioids increases the risk of respiratory complications postoperatively (Taylor et al. 2005).

A recent study evaluated several methods used to assess pain and determine if differences existed between younger and older surgical patients in postoperative pain (Gagliese et al. 2005). These methods included the numeric rating scale (NRS), verbal descriptor scale (VDS), McGill pain questionnaire (MPQ), and the visual analog scale in both the horizontal and vertical (VAS-H, VAS-V) format. Pain intensity did not differ between the age groups, however, the elderly scored lower on the MPQ and self-administered less morphine by patient-controlled analgesia (PCA). The MPQ is comprised of 20 categories of adjectives that describe the sensory, affective, and evaluative pain experience. The elderly used words that described a less intense pain

experience but their scores on the NRS, VDS, VAS-H and VAS-V were not significantly different from the young subjects. Moreover, this study demonstrated that the elderly had difficulty using the Visual Analog Scales (VAS) (Gagliese et al. 2005; Kremer, Atkinson, and Ignelzi 1981) producing high rates of unscorable data and that resulted in low face validity of the VAS method. Consequently, use of the VAS in elderly postoperative patients was discouraged (Gagliese et al. 2005).

As the population of individuals over the age of 65 increases, reservations to perform surgeries based solely on chronological age are decreasing (Farnham et al. 2004). However, postoperative pain management remains grossly inadequate in the elderly. This outcome is mostly due to a limited knowledge about the effects of aging on pain. Better understanding of differences in pain perception, pain processing, primary and secondary sensitization, endogenous pain systems, and underlying psychosocial/cognitive factors between the elderly and young individuals may provide more effective means of managing postoperative pain.

Effect of age on primary sensitization and primary hyperalgesia.

Primary afferent neurons in the peripheral nervous system (PNS) have nociceptors that respond to stimuli that may result in tissue damage. The three types of sensory fibers are Aβ, Aδ, and C. Aβ fibers are large diameter, myelinated, and have nociceptors that respond to innocuous stimuli from skin, muscle, and joints. Aδ fibers are smaller diameter, myelinated, and have nociceptors that respond to noxious heat and mechanical stimuli. These neurons are responsible for acute, sharp initial pain with injury. C fibers are small diameter, unmyelinated, and have nociceptors that respond to thermal and mechanical stimuli. Some nociceptors on C fibers are insensitive to mechanical stimuli, responding only to noxious thermal stimuli. Most C fibers also

respond to noxious chemical stimuli, e.g. acid, capsaicin, and protons. C fiber activation results in delayed, aching, burning pain that accompanies injury.

Nociceptive inputs from the peripheral first order afferent neurons synapse on second order neurons in the dorsal horn which ascend the spinal cord to synapse in the periaqueductal gray and thalamus. Numerous areas of the cortex receive inputs from the thalamus via third order neurons including the somatosensory area II (SII), inferior and anterior parietal cortex, insular cortex, anterior cingulate cortex, and medial prefrontal cortex (Casey 1999; Davis et al. 1997).

Primary afferent neurons become sensitized with tissue injury. Numerous mediators released from nerve terminals and damaged cells, e.g. substance P (SP), glutamate, bradykinin, protons (H^+), interleukins, and histamine can sensitize nociceptors. Characteristics of sensitization include lower response threshold, increased response magnitude to suprathreshold stimuli, increase in spontaneous neuronal firing, and an increase in receptive field size. Peripheral sensitization leads to primary hyperalgesia, characterized by exaggerated responses to elicited pain at the site of injury. Components of primary hyperalgesia include mechanical hyperalgesia, an exaggerated response to a painful stimulus and allodynia, sensation of pain from a normally innocuous stimulus.

Many changes occur with aging that affect pain processing by the PNS. With progression of aging, the number and density of myelinated and unmyelinated nerve fibers were shown to decrease or the features of neuronal degeneration appeared (Ceballos et al. 1999). Morphological changes in neurons associated with aging in mice include a decrease in size, circularity (ratio between measured axonal area and area of a circle with same circumference), and myelin thickness. Nerve conduction velocities are lower and latencies longer in older

animals than younger (Chase et al. 1992; Dorfman and Bosley 1979; Verdu, Buti, and Navarro 1996). These age-dependent changes in neuronal conductivity may be due to segmental demyelination (Adinolfi et al. 1991) or a decrease in axon diameter (Chase et al. 1992). Axonal transport is required for anterograde transport of proteins synthesized in the soma to the axon terminal and for retrograde transport of trophic factors, e.g. nerve growth factor from the terminal to the soma to promote neuronal growth and survival. Rate of axonal transport is reduced with age (Stromska and Ochs 1982) and consequently, can impart cell function.

Changes in neurons caused by aging impact neuronal function and may impede Wallerian degeneration, axonal regeneration, reinnervation of the target organ, and axonal maturation after nerve transection or crush injury (Verdu et al. 2000). Functional, morphologic, and cellular changes in neurons may influence the course of postoperative pain in the elderly. Delay in regeneration and reinnervation in the elderly may be a significant factor contributing to longer-lasting pain and extended convalescence after surgery.

Effect of aging on central sensitization and secondary hyperalgesia.

In addition to changes in the PNS, changes in pain processing occur within the CNS in the elderly. Many Aδ and C fibers synapse on second order neurons, wide dynamic range (WDR) or high-threshold (HT) neurons, in the dorsal horn of the spinal cord releasing excitatory amino acids aspartate and glutamate and neuropeptides including SP, calcitonin gene-related peptide (CGRP) and others. WDR neurons respond in a graded fashion to noxious and innocuous stimuli. The convergence of inputs from Aβ, Aδ, and C fibers allow integration of net input and encode stimulus intensity. Increased nociceptive input can enhance the response or amplify the signal of

WDR neurons in the CNS. HT neurons respond to potentially tissue-damaging stimuli including thermal, mechanical, and noxious chemical stimuli.

Peripheral sensitization of nociceptors increases spontaneous discharge activity. The continuous spontaneous discharges from sensitized Aδ and C fibers in addition to intense, intermittent inputs from nociceptors converge on WDR neurons which, in turn, respond with an amplified or enhanced response to these inputs resulting in central sensitization. Characteristics of central sensitization include: 1) increased receptive field size, afferent input from the area surrounding the injury evoke a painful response (secondary hyperalgesia), and 2) low-threshold tactile stimulation via Aβ fibers activate dorsal horn neurons causing pain (allodynia).

Age-associated changes occur in the dorsal horn of the spinal cord. Thermal withdrawal latencies were significantly shorter in aged than adult rats (Iwata et al. 2002). The WDR neurons of aged rats had significantly larger receptive fields with graded responses to high and low threshold mechanical stimulation and graded heat stimulation (Iwata et al. 2002). Responses of WDR neurons to heat stimuli were significantly greater in aged rats than in adult rats. Furthermore, aged rats had significantly larger receptive fields of nociceptive-specific neurons than adult rats. WDR and HT neurons exhibited significantly higher background activity with more frequent afterdischarges following cessation of noxious stimuli in the aged animals. Differences between aged and adult rats were further examined in a chronic inflammatory state induced by complete Freund's adjuvant (CFA) (Kitagawa et al. 2005). In adult rats, responses of HT neurons were heightened to strong mechanical stimulation and pinch stimuli after CFA treatment, whereas HT neurons in aged rats did not show an increased response. There were no significant differences between these two age groups in response of WDR to mechanical

stimulation after inflammation. The authors stated that aged rats appeared to be in a chronic, hyperexcitable inflammatory state.

While many studies have examined effects of aging on spinal cord injuries, few studies have examined effects of aging on dorsal horn neurons after surgical incision (Goettl et al. 2003; Ma and Bisby 2000; Ohara et al. 1994). Mechanisms of incisional pain may differ from inflammatory, neuropathic, or capsaicin-induced pain. Further investigation of the mechanisms of central sensitization and differences between young and aged may identify more effective methods to manage postoperative pain in the elderly.

Effect of aging on endogenous pain systems.

Descending pathways (opioid-dependent and non-opioid-dependent) from the CNS alter nociceptive processing in the dorsal horn. Nociceptive afferent activity can activate the periaqueductal gray (PAG) or raphe nuclei. The PAG is functionally and anatomically sectioned into a ventrolateral and lateral column. Deep somatic noxious stimuli from muscles, joints, and viscera result in evoked cessation of spontaneous activity, animals become hyporeactive, hypotensive, and bradycardic (Bandler and Shipley 1994). These characteristics are associated with opioid analgesia and the pattern resembles a reaction to injury. Activation of the lateral column from cutaneous noxious stimulation results in a confrontational response, a defensive reaction either fight or flight, hypertension, tachycardia (Bandler and Shipley 1994). These changes are associated with non-opioid analgesia via activated serotonin and noradrenergic pathways. Stress, including pain, can activate the hypothalamic-pituitary-adrenal axis resulting in analgesia. Release of β-endorphin and corticotropin releasing hormone (CRH) produce antinociceptive effects (Hargreaves, Dubner, and Costello 1989; Song and Takemori 1990).

Aging is associated with fewer serotoninergic and adrenergic neurons in the dorsal horn (Iwata et al. 2002). Blockade (local anesthetic) of descending inhibitory pathways in adult rats resulted in increased neuronal responses to heat stimulus (Iwata et al. 2002). However, in aged rats the spinal block did not have an effect on neuronal responses. This suggests that descending inhibitory neurons to nociceptive afferents in aged rats are less effective in suppressing nociceptive inputs than in adult rats. Distribution of serotonin and dopamine-β-hydroxylase (DBH) in the dorsal horn was sparse and the area occupied by immunoreactive serotonin and DBH fibers was significantly smaller in aged than adult rats (Iwata et al. 2002). Serotonin and DBH immunoreactive neurons were also smaller with greater aberrant morphology seen with degenerative changes. In a study to assess the endogenous descending inhibitory mechanisms, elderly and young adult volunteers were tested by immersing their hand into cold water (Washington, Gibson, and Helme 2000). Pain thresholds were increased in both groups however, the magnitude of analgesic response induced by cold water immersion was significantly lower in the elderly. Both groups tolerated higher pain thresholds after the cold immersion test. The young attained a 100% increase in electrical pain threshold from baseline whereas the elderly exhibited a 30% increase in electrical pain threshold from baseline.

The deterioration of descending inhibitory neurons in the elderly may lead to altered pain perceptions from surgical incision and tissue manipulation. Decreased inhibition of afferent inputs may result in greater pain perception resulting in enhanced peripheral and central sensitization. Postoperative pain animal models should be used to further characterize changes in the function of descending inhibitory neurons during aging.

Effect of aging on psychosocial/cognitive aspects of pain.

Pain is comprised of two components: sensory-discriminatory and cognitive-affective. The sensory-discriminatory component refers to ability of the nervous system to sense noxious stimuli and determine intensity. The cognitive-affective component determines the reaction by the individual or animal to the noxious stimulus. Elderly are less likely to label a noxious stimulus as unpleasant, and exhibit greater stoicism (Chakour et al. 1996; Washington, Gibson, and Helme 2000). Higher anxiety levels correlated with higher VAS scores and ratings of pain as unpleasant; anxiety was the only psychometric parameter that negatively correlated with age, suggesting elderly subjects displayed lower levels of anxiety (Chakour et al. 1996). However, in a study by Washington (Washington, Gibson, and Helme 2000) no significant difference in anxiety between the young and elderly subjects were found. Despite reporting similar pain intensities on NRS or VAS, elderly tend to report lower MPQ scores, notably, within the subcategories of total and sensory pain rating indices (Gagliese and Katz 2003). As compared to the elderly, young patients tend to select more words and of greater intensity, e.g. burning or shooting, to describe low back pain.

Nociception is not the only sensory modality involved in pain sensation. In tests of mechanical pain tolerance, higher cognition scores corresponded to greater pain tolerance (Pickering et al. 2002). This study also demonstrated that mechanical pain tolerance and detection were associated with faster reaction time and with greater auditory sensitivity. Anatomical and physiological changes that occur with aging impact a person's ability to integrate information from multiple sensory modalities and interpret the information.

Consequences of unrelieved postoperative pain in the elderly

Moderate to severe postoperative pain can affect nearly every organ function and impact postoperative morbidity and mortality. The elderly population is at greater risk for perioperative complications due to an increase in comorbidities and a decline in organ function that accompanies aging. Acute surgical pain elicits a neuroendocrine stress response (Brodner et al. 2001) which is proportional to the magnitude of surgical trauma. Levels of catabolic hormones, e.g. catecholamines and cortisol, increase and anabolic hormone, e.g. insulin, decrease resulting in a negative nitrogen balance and increased lipolysis (Kataja et al. 2007). Increases in renin, aldosterone, angiotensin, antidiuretic hormone, and cortisol result in sodium and water retention (Monk, Mueller, and White 1992). Reduction of the magnitude of stress response to surgery is critical for the elderly patient and can be accomplished by effective intra- and postoperative multimodal analgesia techniques (Holte and Kehlet 2002).

Surgical stress by activation of the sympathetic nervous system leads to hypertension, tachycardia, increased systemic vascular resistance and greater myocardial irritability increasing the risk of myocardial ischemia or infarction (Mangano et al. 1991). Age-related changes in cardiovascular function interfere with the ability of elderly to compensate for increased sympathetic nervous system activity. Increased peripheral vascular resistance, increased systemic blood pressure and an enhanced propensity for arrhythmias along with decreased coronary artery flow, decreased arterial elasticity and a reduced ejection fraction leaves a narrow window for aged patients to handle the hemodynamic challenges of surgical pain-induced sympathetic nervous system activation (Cook and Rooke 2003).

Pain after surgery limits mobility compromising pulmonary function by reducing ability to cough and deep-breathe effectively. The result is an increased potential for atelectasis, decreased arterial oxygenation, retention of carbon dioxide, and infection (Zaugg and Lucchinetti 2000). Elderly experience age-related changes in pulmonary function, e.g. decreased vital capacity, reduced chest excursion and a blunted ventilatory response to hypoxemia or hypercarbia (Zaugg and Lucchinetti 2000). These changes compounded with the effects of inadequately treated pain increase the potential for deleterious outcomes in the elderly patient.

Sympathetic nervous system activation increases sphincter tone and decreases intestinal motility (Liu et al. 1995; Smith, Kelly, and Weinshilboum 1977) Opioid analgesics given for the relief of pain often intensify these effects resulting in ileus, nausea, vomiting, and constipation. In the elderly patient, where nutritional status may be compromised prior to surgery, recovery is further hampered by poor grastrointestinal function. Diminished renal reserve in the elderly increases susceptibility to acute renal failure in the perioperative period further reducing the glomerular filtration rate and elimination of metabolites (Epstein 1996). Decreasing renal function with age can have significant effects on drug pharmacokinetics and clearance. Pharmacokinetics of commonly administered opioid analgesics, e.g. morphine, are altered in the elderly patient. Decreases in lean body mass, decreased cardiac outputs, reduced metabolism and renal clearance of metabolites warrant individualized titration of drugs to the desired effect while minimizing potentially deleterious side effects.

Inadequate postoperative pain management has been related to increased incidence of postoperative delirium in the elderly (Dyer, Ashton, and Teasdale 1995; Lynch et al. 1998; Rosenberg, Rosenberg-Adamsen, and Kehlet 1995). Higher levels of pain at rest, but not with movement, were associated with increased risk of delirium over the first 3 postoperative days. In

addition to pain, an increase in postoperative delirium or cognitive dysfunction has been related to advanced age, lower education status (Grichnik et al. 1999), pre-existing dementia, previous alcohol abuse, low albumin and anemia (Robinson et al. 2009).

Controlling pain in the elderly patient reduces risk of inducing the stress response and potentially deleterious cardiovascular effects, improves pulmonary function and reduces incidence of atelectasis or pneumonia, and reduces a negative impact on cognition.

Experimental models of acute postoperative pain

The mechanism of postoperative pain has gained interest since the mid-1990s. Many studies have characterized changes in nociception with aging with or without pharmacologic agents. Incisional pain models have been used to examine changes in nociception, behavior, and neuronal plasticity. The efficacy of drugs, alone and in combination, e.g. opioids, non-steroidal anti-inflammatory drugs (NSAIDs) and local anesthetics, were also studied using the incisional pain models. However, none of the incisional models have been used to characterize the impact of aging on postoperative pain. Table 1 presents a comprehensive summary of various pre-clinical experimental models used to evaluate different aspects of postoperative pain. These models are organized into four main categories: chest, skin/fascia/muscle, orthopedic/neurological procedures, and viscera. Each category includes different surgical manipulations. Pain assessment methods used in these models are provided along with general descriptors of features addressed by each of these models.

Chest. Two thoracotomy models have been described. To address the problem of chronic pain after thoracotomy, Buvanendran and colleagues (Buvanendran et al. 2004) developed a model for postthoracotomy pain. This model produced a 50% rate of allodynia

which was similar to the rate observed in humans. The authors postulated that the nerve injury was likely due to pressure on the nerve from the retractor resulting in ischemic or compression injury. Damaged neurons from animals that developed allodynia showed extensive Wallerian degeneration with few intact fibers and remyelinated axons with a small diameters and thin myelin sheaths. Elderly may be at greater risk for postoperative allodynia due to morphologic (e.g., decrease in size, circularity, and myelin thickness) (Ceballos et al. 1999), and intracellular (e.g., reduced rate of axonal transport) changes that occur with aging (Stromska and Ochs 1982). This model could be employed to evaluate differences in postthoracothomy pain between young and aged rats.

Skin/fascia/muscle. Several less complex models have been developed to investigate mechanisms of postoperative pain resulting from skin, fascial and/or muscle afferent input. The plantar paw model is well characterized and has been used extensively to determine mechanisms of primary and secondary hyperalgesia (Banik and Brennan 2004; Brennan, Umali, and Zahn 1997; Brennan, Vandermeulen, and Gebhart 1996; Carnell 1999; Koizuka et al. 2005; Leonard, Arunkumar, and Brennan 2004; Zahn, Pogatzki-Zahn, and Brennan 2005). Benefits of this model include the relatively simple surgery and primary and secondary hyperalgesia that typically lasts approximately five days. Animals do not exhibit behaviors associated with intense pain such as vocalization or flinching. Testing modalities include response to punctate mechanical stimulus (von Frey filaments) and assessment of non-evoked behavior (paw position on or off mesh cage). However, most surgical incisions occur on hairy skin and differences exist between nociceptive inputs from hairy (dorsum) versus glabrous (plantar paw) skin (Granovsky et al. 2005; Rendell et al. 2002). The dorsal, hairy skin model of postincisional pain was developed to more closely reproduce changes that occur in human surgery (Duarte et al. 2005).

The investigators developed a graded response to characterize vigor of contractile response to von Frey filament application and compared these to a population response, an all-or-none response. Primary (adjacent to incision) graded responses to allodynia resolved faster than hyperalgesia while both remained significantly greater than baseline for the entire postincisional week. Secondary (distant from incision) graded responses were smaller and resolved faster than primary graded responses. Population response scores increased faster and persisted the entire week compared to graded response which increased gradually over 3-4 hours, decreased by more than half peak value at one week or insignificant by 72 hours for primary and secondary allodynia, respectively. This model provides a method to quantify allodynia and hyperalgesia and to spatially separate them as primary and secondary. Mechanisms of allodynia and hyperalgesia may correspond to different cutaneous fiber types and possibly, different mechanisms of sensitization. In a study to evaluate differential effect on C fiber-mediated pain versus pain perception mediated by Aδ fibers, young adults exhibited significant increases in thermal pain threshold during A fiber block while pain threshold remained relatively stable during the testing period in the elderly group (Chakour et al. 1996). This suggests elderly rely primarily on C fiber inputs for pain perception while young adults perceive pain through both Aδ and C fibers. Use of the hairy skin model would allow delineation of differences in hyperalgesia and allodynia between young and elderly groups.

Orthopedic/neurological procedures. Evaluation of postoperative pain associated with orthopedic procedures has been addressed in several models including a hole drilled in the tibia or calcaneus (Houghton, Hewitt, and Westlund 1997), a model of knee surgery (Buvanendran et al. 2008), laminectomy with disc injury (Massie et al. 2004), and tibia fracture with internal fixation (Minville et al. 2008). While most of these models use rats, the tibia fracture with

internal fixation utilizes a mouse model. Many orthopedic procedures are performed on geriatric patients. Elderly are susceptible to falls and other traumatic injury with age due to loss of muscle strength, loss of coordination, and reduced capacity of senses (visual, auditory, mechanical pressure) (Kannus et al. 2005; Kim and Robinson 2005). Results from studies on this model may lead to more effective management of postoperative pain in the frail elderly who often suffer from orthopedic injuries.

Visceral. Many surgical procedures involve manipulation of internal organs. Visceral nociceptive inputs may account for general malaise associated with postoperative recovery. Several models have been designed to address the more complex issues of visceral postoperative pain (Lascelles et al. 1995; Liles and Flecknell 1993; Martin et al. 2004; Tong, Conklin, and Eisenach 2006). To investigate the effects of visceral stimulation in conjunction with cutaneous injury by incision, a model for postoperative pain using a subcostal incision was developed (Martin et al. 2004). Prior to surgery, animals underwent operant conditioning to receive sucrose pellets upon pressing a lever in response to a light stimulus. Postoperatively, the rats exhibited less ambulation, rearing, stereotypy, and obtained fewer pellets with less efficiency than sham-operated rats. Less physical activity and decreased desire/ability to obtain food are similar to changes seen in the clinical setting after abdominal surgery in humans (Martin et al. 2004). Two of the most common complications after abdominal surgery are impaired gastrointestinal function (nausea, vomiting, ileus) (Fevang et al. 2000) and respiratory depression (Taylor et al. 2005). Advanced age and co-morbidities were associated with both of these complications (Fevang et al. 2000; Taylor et al. 2005). This model may be used to develop more effective postoperative management of elderly after abdominal surgery to reduce complications.

Tong and co-investigators (Tong, Conklin, and Eisenach 2006) developed an interesting model combining a laparotomy with cervical distension to address the visceral component to surgical pain, inflammation and somatic pain. Rats subjected to the laparotomy with cervical distension exhibited a significant reduction in vertical activity, decreased feed and drinking behavior, and a unique 'squashing' behavior. The squashing behavior is believed to result from visceral pain induced by the cervical distension. This model could be utilized to study gender/sex differences and effect of age on postoperative pain. Young women report greater pain intensities than men but this gender difference disappears in elderly subjects (Aubrun 2005; Pickering et al. 2002).

Application of the dorsal hairy skin incision model of postoperative pain in aged rats.

To date, no research addressed the effects of aging on postoperative pain. None of the developed experimental models was adapted to study post surgical pain and aging. This is a major limitation to the progress of research on the impact of age on postoperative pain. Recently, the dorsal hairy skin incision model has been successfully adapted in our laboratory to characterize postoperative pain in young adult (5-7 months) and aged (22-24 months) male Fischer 344 rats. Paraspinal dorsal incision was made on hairy skin under anesthesia. After recovery, graded responses of *cutaneous trunci* muscle reflex were tested with von Frey filaments. Three measures of pain were assessed: primary hyperalgesia, adjacent to the incision; ipsilateral secondary hyperalgesia, 2 cm from incision on the same side; and contralateral secondary hyperalgesia, 2 cm from incision on the opposite side of the spinal column. Measurements were made at 5 baseline sessions and then postincision at 3 hr, 6 hr, and days 1, 3, 6, 8, 10 and 14. Nociception testing included primary and secondary (ipsilateral and

contralateral) hyperalgesia. Baseline responses to von Frey filaments (0.07 – 26 g) were significantly higher in aged rats than young rats. The results from this study showed that the aged rats did not develop primary hyperalgesia in response to surgical incision. Secondary hyperalgesia was absent after surgery in both aged and young rats. Notably, in this model, young and aged rats developed sensitization to the testing by von Frey filaments over time. Aged rats had higher initial baselines, and exhibited greater variability in response than the young rats. The absence of hyperalgesia after surgical incision in aged rats may be partly due to the greater sensitivity measured during the baseline testing, therefore obscuring any change in cutaneous pain perception in response to incision. This interesting initial finding needs further investigation.

Summary

The increasing population of individuals over the age of 65 and the growing proportion of the 'oldest of the elders' (i.e., individuals over 85 years of age) has a negative impact on healthcare delivery. Many elderly are frail, have co-morbidities, and most take one or more medications. Surgery induces a state of stress in the elderly that renders them less able to maintain homeostasis as compared to younger individuals. Increasing prevalence of dementia in the elderly further complicates the delivery of adequate care. Postoperative management of pain in the elderly patients is inadequate with almost half of the elderly patients reporting moderate to severe pain.

Pain processing is affected by aging. Decreased fiber density, demyelination, and reduced axonal transport are but a few changes associated with aging and may prolong postoperative pain. Age-related changes within the CNS result in larger receptive fields of WDR

and HT neurons that enhance or magnify pain sensation. Descending inhibitory pathways from the CNS are affected by age-related changes in neuronal density, morphology, and reduced neurotransmitter release. This results in a reduced capacity to inhibit afferent nociceptive input to the CNS. Despite changes in pain processing, the elderly report intensity of pain similar to young adults but report lower ratings of unpleasantness.

Several animal models have been developed to study postoperative pain including types such as orthopedic, nerve injury, abdominal (hairy skin and visceral afferent input), glabrous skin, and hairy skin. Use of experimental postoperative pain models will help to discern how the elderly differ from young. Ultimately, the new knowledge and better understanding of age-related changes in postoperative pain will lead to development of more effective postoperative pain management strategies and reduce morbidity and mortality in the elderly who undergo surgical procedures.

Table 1: Postoperative pain models in rats

Model	**Pain assessment method**	**Focus of the model**
Chest		
Thoracotomy with nerve ligation (Nara et al. 2001)	vFf[a], acetone, forceps	Neuropathic pain
Thoracotomy with rib retraction (Buvanendran et al. 2004)	vFf, acetone, scratching behavior	Neuron damage due to rib retraction resulting in allodynia
Skin; Skin/fascia/muscle		
Dermal tail (Weber et al. 2005)	Mechanical baralgometer, tail withdrawal hot water bath	Primary and secondary hyperalgesia, inflammation at site of incision
Dorsal hairy skin (Duarte et al. 2005)	*Cutaneous trunci* muscle reflex, vFf	Primary and secondary hyperalgesia
Gastrocnemius muscle (Pogatzki, Niemeier, and Brennan 2002)	vFf, blunt disc, radiant heat	Focus on secondary hyperalgesia
Plantar paw (Brennan, Vandermeulen, and Gebhart 1996)	vFf, cumulative pain score (guarding behavior of paw)	Cutaneous/fascial post-incisional pain
Skin/muscle incision & retraction (Flatters 2008)	vFF, pinprick, focused beam radiant heat, acetone	Secondary hyperalgesia, persistent postoperative pain

Orthopedic/neurological procedures		
Hole in tibia or calcaneus (Houghton, Hewitt, and Westlund 1997)	vFf, acetone, hot plate	Primary and secondary hyperalgesia, bone pain
Knee surgery (Buvanendran et al. 2008)	Vertical activity, ambulation, squeeze knee joint	Functional measures of postoperative pain, effect on movement
Laminectomy with disc injury (Massie et al. 2004)	vFf	Concave tail posture, postlaminectomy syndrome pain
Tibia fracture with internal fixation (mouse) (Minville et al. 2008)	vFf, hotplate, subjective pain scale (observed guarding behavior)	Post-trauma model, bone pain, chronic pain, complex regional pain syndrome
Visceral		
Laparotomy with cervical distension (Tong, Conklin, and Eisenach 2006)	Vertical activity, squashing behavior, licking abdomen, grooming, feeding, drinking	Visceral component to surgical pain, inflammation, somatic pain
Laparotomy with common bile duct ligation (Liles and Flecknell 1993)	Ambulation, weight changes, food and water intake	Deleterious effects after surgery due to pain

Laparotomy with intestinal manipulation (Martin et al. 2004)	Vertical activity, ambulation, stereotypy, conditioned operant responding	Pain, cognitive impairment, sedation
Ovariohysterectomy (Lascelles et al. 1995)	Paw pressure test, tail flick latency	Secondary mechanical and thermal hyperalgesia

[a] vFf—von Frey filaments

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THE EFFECT OF AGE ON POSTINCISIONAL PAIN IN FISCHER 344 RATS

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CHAPTER III

THE EFFECT OF AGE ON POSTINCISIONAL PAIN IN FISCHER 344 RATS

Abstract

The age-related changes in postsurgical pain are poorly understood. Consequently, postoperative pain in the elderly is often undertreated and results in increased morbidity. The effects of age on the intensity and duration of postincisional pain were studied in young and aged Fischer 344 rats. A paraspinal dorsal skin incision was made under anesthesia and after recovery graded responses of the *cutaneous trunci* muscle reflex were tested with von Frey filaments. Baseline responses to von Frey filaments were significantly lower in young rats than aged rats. Young rats developed primary hyperalgesia from 3 hours to postincisional day 6 whereas aged rats did not develop significant postincisional primary hyperalgesia. Secondary hyperalgesia was absent after surgery in both young and aged rats. Data from the experimental postoperative pain model suggested that in rats, aging resulted in a significantly reduced threshold to mechanical nociceptive stimulus and lack of primary and secondary hyperalgesia. The absence of hyperalgesia in aged rats may be related to the greater baseline sensitivity, therefore obscuring any change in cutaneous pain perception in response to incision. Perspective: The mechanism of postincisional pain may differ between young and aged adults. An adequate experimental model of postsurgical pain applied to aged rats could help generate findings that can lead to more effective methods for treatment of postoperative pain in the elderly.

Introduction

Greater numbers of adults are having surgical procedures at advanced age. Once a criteria for exclusion, the age of 80 years is no longer a contraindication to invasive surgical procedures, e.g. pancreaticoduodenectomy (Sohn et al. 1998), total knee replacements (Biau et al. 2006), or video-assisted thoracic surgery for lobectomy (McVay et al. 2005). Despite the growing numbers of elderly undergoing surgery, treatment of their postoperative pain remains inadequate (Karani and Meier 2004; Lynch et al. 1997; Maxam-Moore, Wilkie, and Woods 1994; Morrison and Siu 2000). Additional difficulty with management of postoperative pain in the elderly is related to the significantly increased susceptibility to side effects from opioid analgesics (Taylor et al. 2005). Consequences of inadequately treated postoperative pain such as compromised pulmonary function (Richardson et al. 1994), activation of the sympathetic nervous system (Breslow et al. 1989), delirium (Duggleby and Lander 1994), and increased length of hospital stay (Bapat et al. 2005) lead to increased morbidity and mortality.

Knowledge of the effects of age on postoperative pain is limited. Many studies report no significant differences in visual analog scale (VAS) scores for young vs elderly patients undergoing similar surgical procedures (Aubrun et al. 2003; Aubrun et al. 2002; Gagliese and Katz 2003; Kaiko 1980). It was reported that elderly tend to consume less postoperative analgesics (Macintyre and Jarvis 1996; Moore et al. 1990; Woodhouse and Mather 1997), however, other studies showed no significant differences in consumption of postoperative analgesics (Aubrun et al. 2003; Aubrun et al. 2002). Very few studies focused on investigating changes in postsurgical pain during the aging process and their experimental approaches differ significantly.

Injury induced by surgical incision and tissue manipulation results in primary sensitization of peripheral nociceptors (Pogatzki, Niemeier, and Brennan 2002; Zahn and Brennan 1999a). The constant barrage of inputs from the periphery induces central sensitization in the spinal cord dorsal horn neurons manifested as secondary hyperalgesia (Pogatzki, Niemeier, and Brennan 2002; Zahn and Brennan 1999a). Thus, postoperative pain is accompanied by primary and secondary hyperalgesia. Primary sensitization refers to sensitization of peripheral afferent fibers and is characterized by a lower response threshold, an increased response magnitude to suprathreshold stimuli, increased spontaneous activity and increased receptive field size (Campbell et al. 1988; LaMotte et al. 1982; Thalhammer and LaMotte 1982). These changes result in primary hyperalgesia, an exaggerated response eliciting pain at site of injury. The increase in afferent impulse frequency and magnitude induce neuronal plasticity in the dorsal horn neurons (Menetrey and Besson 1982). This plasticity, central sensitization, modifies nociceptive pathways by enhancing and prolonging the responses to subsequent peripheral stimuli. Central sensitization plays a major role in heightened pain sensitivity after peripheral injury. It is responsible for the pain produced after injury by normally innocuous low threshold afferent inputs and the spread of hypersensitivity to regions beyond injured tissue (Woolf and King 1990). The areas of hypersensitivity may exhibit characteristics of allodynia, the perception of pain to a normally innocuous stimulus and hyperalgesia, an exaggerated perception of pain to a normally noxious stimulus.

Age-related changes in the peripheral and central nervous systems must be presumed to alter pain processing and perception postoperatively, yet to date no animal models have been developed to study the specific mechanisms underlying the effect of age on postoperative pain. We addressed this need in this study by adopting, with modifications, the dorsal skin incision

model (Duarte et al. 2005; Mujenda et al. 2007) to not only young but also aged rats using von Frey filaments at varying forces for sensitivity testing. The goal of this project was to identify age-related differences in duration and intensity of postincision allodynia and hyperalgesia in male Fischer 344 rats.

Methods

Animal handling and preparation for testing

Two groups of male Fischer 344 rats, young aged 5-7 months (n=17) and aged 22-23 months (n=16) obtained from the National Institute on Aging were used in this project. Rats were housed in pairs in clear polycarbonate cages in the University of North Texas Health Science Center vivarium at room temperature 22-24°C, under a 12-hour light/dark cycle, with lights on at 0700 h. Rats were given *ad libitum* access to food and water. After the last testing period, rats were euthanized by decapitation. All procedures involving rats were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of North Texas Health Science Center at Fort Worth.

Nociceptive testing procedures were adopted from Duarte and colleagues (2005) and Mujenda and colleagues (2007) and modified to the model of aged rat. Rats were handled for five 5-min sessions over three days to acclimate the animals to the experimenter and testing environment. During the acclimation sessions, rats remained still on the experimenter's forearm with gentle guiding to tuck rat nose into crook of experimenter's arm.

In preparation for sensitivity testing, the day before the first testing, the specific dorsal skin area was prepared by clipping the fur and marking specific skin testing areas with indelible markers. The incision site was marked parallel to the spinal column 1 cm to the right beginning

at the iliac crest and extending rostrally 2 cm. The ipsilateral and contralateral testing areas were marked 2 cm from the incision site avoiding bony prominences. These marked dorsal skin areas were used for sensitivity testing in five (5) baseline sessions before incision and then, in a series of different time points after the incision (see details in next section).

Surgical incision

The surgical procedure was adapted from Duarte et al. (2005) and Mujenda et al. (2007) with modifications. Before the surgical procedure, the fifth baseline test was performed. After baseline testing, anesthesia was induced with isoflurane at 5% until cessation of movement then continued with 2-3% isoflurane via nose cone. Heart rate and oxygen saturation was monitored with pulse oximetry. The skin was prepped with povidone-iodine scrub, alcohol, followed by povidone-iodine solution. After ensuring adequate anesthetic depth, a 2 cm incision was made through the skin with a blade (#10) leaving the underlying fascia intact. The incision was closed with three sutures using the 4-0 prolene. During the surgical procedure and recovery the rats were placed on a heated plate (39°C) covered with a soft, absorbent pad. The surgical incision was tolerated well by the animals; no change in grooming behavior, ambulation, weight loss, or wound infection was observed. Control animals were subjected to all procedures as the surgical group but did not undergo skin incision. Rats were housed singly after surgery.

Testing of sensitivity to von Frey filaments

Cutaneous trunci muscle reflex (CTMR) (Theriault and Diamond 1988) to von Frey filaments stimulation was recorded. Thirteen (13) filaments, force range from 0.07 g to 26 g, were tested sequentially beginning with the area marked 0.5 cm from the incision (primary

allodynia/hyperalgesia), followed by testing on the area 2 cm from incision, right of spinal column (ipsilateral secondary allodynia/hyperalgesia), and then followed with testing the area 2 cm from incision, left of spinal column (contralateral secondary hyperalgesia). Each filament was briefly pressed to the skin until the filament bent and then it was removed. Filaments were applied four times at 2- to 3-second intervals. The CTMR graded responses were recorded as 0 (no reflex), 0.5 (small reflex), or 1 (large reflex) as previously described (Duarte et al. 2005; Mujenda et al. 2007). For each testing session, the four responses to a filament were averaged and recorded as the response to the filament. Five baseline tests were conducted over 5-6 days; the fifth baseline response was recorded before the incision. The last three tests results were averaged and reported as baseline values. The postincision (postoperatively) graded responses were recorded at 3h, 6h, and then daily on postoperative day (POD) 1, 3, 6, 8, 10, and 14. Based on baseline graded response in the primary hyperalgesia test area, rats were matched for sensitivity and placed accordingly into control and incision groups.

A response to low force filaments, which prior to surgery did not elicit a response, was defined as allodynia. Increased responsiveness, i.e. a greater CTMR graded response to a filament after surgery, was defined as hyperalgesia. An increased response to von Frey filament stimulation over time was defined as sensitization.

Statistical analysis

Data are presented as the mean ± S.E. for each filament for separate groups of 8-9 rats per group. To determine the effects of incisional surgery on graded response, data were analyzed by 2-way ANOVA with Treatment and filament Force as between group factors. The effect of time on graded response before and after incision was determined by analyzing the data by 3-

way ANOVA with Surgery and filament Force as between group factors and Time as a within-group factor. To determine the effect of age on graded response to categories of filaments (low, mid, and high), data were analyzed by 2-way ANOVA with Age and Incision as between group factors. Lastly, the effect of age on graded response in baseline testing data was analyzed by 3-way ANOVA with Age (n=17 young; n=16 aged) as between group factors and Region and Session within-group factors. Planned individual comparisons among Age, Treatment, and Time were performed using single degree-of-freedom F tests within the 3-way interaction for each set of data. Planned individual comparisons for baseline data were performed among Age, Region, and Session using single degree-of-freedom F tests within the 3-way interaction. Statistical significance was defined as $p < 0.05$.

Results

Effect of incision on sensitivity to von Frey filaments (graded response)

The effect of incision on the CTMR graded response to von Frey filaments (0.07 – 26 g) in young and aged rats is presented on Figure 1. Young rats demonstrated allodynia at 3 hours postincision with the 0.6 g filament ($p = 0.05$, Figure 1, left panel) and primary hyperalgesia beginning at 3 hours with fibers 1, 1.4, 2, and 4 g ($p < 0.049$), increasing by POD 1 with fibers 6, 8, 10, 15, and 26 g ($p < 0.009$). Graded responses were elicited by these fibers in baseline testing and the CTMR graded response increased after incision. Primary hyperalgesia in the young rats returned to baseline by POD 8. In contrast, aged rats did not develop significant allodynia or primary hyperalgesia at any point during the testing period after the incision (Figure 1, right panel).

Rats were matched for sensitivity of graded response based on the primary hyperalgesia region for allocation to control or incision groups. The aged rat control group was not well matched for ipsilateral and contralateral secondary hyperalgesia due to regional differences in sensitivity (Figures 2 and 3). During the postincision testing period with all 13 filaments, ipsilateral secondary hyperalgesia and contralateral secondary hyperalgesia did not develop at any time point for any filament force in either young or aged rats. Baseline and 6 hour postincision graded responses in Figure 2 illustrate a lack of ipsilateral or contralateral secondary hyperalgesia development. The control aged rat group had significantly higher baseline responses and higher postincision responses measured at 6 hr when compared to the surgical group in both ipsilateral and contralateral secondary hyperalgesia areas (secondary ipsilateral, baseline, $p = 0.008 – 0.033$; secondary ipsilateral, 6 h, $p = 0.019 – 0.029$; secondary contralateral, baseline, $p = 0.005 – 0.043$; and secondary contralateral, 6h, $p = 0.022 – 0.045$; Figures 2 and 3). Across the entire testing period and the range of von Frey filaments, graded responses in aged rats exhibited greater variability than in young rats**.**

Figure 4 presents time course data for 0.6 g, 2 g, and 10 g von Frey filaments. Each filament time force is representative of a low, mid, or high force filament. Young rats exhibited significant primary allodynia at 3 hours ($p = 0.046$) and POD 1 ($p = 0.014$) in response to the 0.6 g filament. Young rats developed significant primary hyperalgesia by the 3rd hour that lasted through POD 6 in response to the 2 g ($p = 0.002 – 0.033$) and 10 g ($p = 0.007 – 0.038$) filaments. In general, the aged rats had greater graded responses to all three filaments compared to the young rats. However, the aged rats did not develop primary hyperalgesia in response to any of the three representative filaments. Aged control rats had a significantly greater graded response than aged postincision rats on POD 1 ($p = 0.046$).

Age-related differences in response to nociceptive testing by von Frey filaments were expressed as force-response curves by fitting the mean CTMR graded response to a 4-parameter logistic dose response function of filament force. In the analysis of the effect of age on graded responses, the von Frey filaments were grouped into three force categories based on shape of the sigmoidal force-response relationships obtained for the young rats (Figure 1). The group of the lower force filaments from 0.07 g to 0.6 g, corresponded to the flat portion of the curve. The steep slope of the curve corresponded to the mid-range filament forces, 1-6 g. The plateau of the force-response curve corresponded to the high force filaments from 8 g to 26 g. Elicitation of the CTMR in response to the lowest force filaments postincision is indicative of allodynia. An increase in response to either the mid and/or high force filaments indicates hyperalgesia. Figure 5 shows primary allodynia/hyperalgesia within each category of the filament forces for baseline, 3 hours postincision, POD 1, and POD 8.

At the baseline, aged rats (control and surgery) had significantly greater graded responses than young control rats at the mid ($p < 0.009$) and high ($p < 0.001$) filament forces (Figure 5). At 3 hours postincision and on POD 1, aged rats showed significantly higher graded responses than young controls in each of the three filament force categories (3h: low, $p = 0.011 – 0.023$; mid, $p < 0.001$; high, $p = 0.002 – 0.007$; POD 1: low, $p < 0.049$; mid, $p < 0.001$; high $p < 0.001$). On POD 8, aged rats had significantly greater graded responses at low (control only, $p = 0.001$) and mid forces (control and incision, $p = 0.001$) compared to young control. At each tested time, there were no statistically significant differences between aged control and aged incision rats. Significant primary hyperalgesia was measured in the mid ($p = 0.002$) and high ($p < 0.002$) forces at 3 hours after incision and on POD 1 in young incision rats. Although allodynia was

measured in young rats at 3 hours postincision and POD 1, in the low force filament group the differences were not statistically significant.

Age-dependent differences in the ipsilateral and contralateral secondary testing areas at the baseline are shown on Figure 6. Aged rats, control and incision, had significantly higher graded secondary ipsilateral responses in the low ($p < 0.001$), mid ($p < 0.001$), and high ($p < 0.001$) force group, and higher contralateral responses in high ($p < 0.001$) filament force category as compared to young control. In the contralateral area, the aged control group had higher graded responses at the low and mid filament forces ($p = 0.001$) than young control.

Effect of age on sensitization during baseline testing

During the five baseline testing sessions, the young and aged rats developed a progressive increase in graded response (Figure 7). The data from control and incision groups were combined since handling of rats in both groups during baseline testing period was identical. Baseline graded responses were significantly higher in aged rats than in young rats for all three areas tested (primary, $p < 0.017$; ipsilateral secondary, $p < 0.001$; and contralateral secondary, $p < 0.001$) and continued to increase over time. The greater baseline values in the aged rats indicate greater sensitivity, i.e., greater responsiveness to nociceptive stimuli. Sensitization was present in young and aged rats. Based on the 3-way ANOVA, there was no significant interaction of Age, Session, and Region (primary, ipsilateral secondary, or contralateral secondary test regions). There was a significant interaction ($p < 0.001$) of Age and Region indicating that age-related differences in graded response exist among the three tested regions. In aged rats, the ipsilateral secondary region showed significantly greater sensitivity (greater graded response) than the primary or contralateral secondary regions. There was no significant

interaction of Age and Session suggesting that there were no significant age-related differences in sensitization over the five testing sessions.

Discussion

The findings from this research project demonstrate that in male Fischer 344 rats, aging results in significantly reduced threshold to mechanical nociceptive stimulus and lack of primary and secondary hyperalgesia. Baseline responses to mechanical stimulation were significantly higher in aged rats than young rats. In contrast to young rats, aged rats did not develop significant primary hyperalgesia after incision. Age differences in primary hyperalgesia appeared to depend on the intensity of the nociceptive stimulus; at lower forces age differences were more apparent. In aged rats, baseline values for secondary hyperalgesia were significantly higher than in young rats. After incision, secondary hyperalgesia was absent in both aged and young rats. These novel findings on the effects of age on postincisional pain were generated on the aged rat model of postoperative pain.

The postoperative pain model used in this research study, previously developed for young rats (Duarte et al. 2005; Mujenda et al. 2007), was adapted to aged rats with several notable modifications. The first change was the rat strain; Fischer 344 rats were selected instead of Sprague-Dawley rats. The aged Fischer 344 rats are commonly used in aging studies because these rats have no excessive weight gain with age (Nadon 2004; Turturro et al. 1999). Comparison of findings from our study on Fischer 344 rats and results from Sprague-Dawley rats reported by Duarte, et al., (2005) showed that the onset and duration of primary hyperalgesia in young Fischer 344 and young Sprague-Dawley rats were similar (Table 1). Strain differences may account for lack of secondary hyperalgesia in Fischer 344 young rats. The second key

difference we made in the aged rat model of postoperative pain was the size of incision; instead of 1 cm we made a 2 cm skin incision. The larger incision afforded a greater surface area for the testing procedure, especially testing of the contralateral secondary hyperalgesia. As shown in Table 1, responses in the primary hyperalgesia region to a 2 g (≈ 18 mN) filament were lower in 1.5-month old Sprague-Dawley rats than in 5-7-month old Fischer 344 rats at baseline (0 vs 0.208), 3 hours postincision (0.38 vs 0.625), and on POD 6 (0.19 vs 0.563). Responses in the primary hyperalgesia region to 10 g (≈ 90 mN) filament were similar for Sprague-Dawley and Fischer 344 rats at baseline (0.55 vs 0.559) and 3 hours postincision (0.88 vs 0.813). For POD 6, the graded response was lower in the 1.5-2-month old Sprague-Dawley than in the 5-7-month old Fischer 344 rats (0.71 vs 0.922). This difference may be related to age, i.e., 2-5 months, between rats used in these two different studies. This age difference is clearly evident within the Fischer 344 rats; young rats have consistently lower graded responses to 2 g and 10 g filaments than aged rats.

The paraspinal dorsal incision model (Duarte et al. 2005) for evaluating postoperative pain in rats was chosen for our study for three main reasons. First, in humans, most surgical procedures are performed on hairy skin, not glabrous. Hairy and glabrous skin types differ in blood flow response to wound healing (Rendell et al. 2002). Development and duration of primary and secondary hyperalgesia is similar in hairy (Kawamata et al. 2005) and glabrous (Zahn and Brennan 1999a) skin rat models. These results correlate with experimental findings from human subjects (Kawamata et al. 2002). Second, the effect of age on development and duration of primary and secondary hyperalgesia in the rat dorsal incision model is not known. A 1-cm dorsal incision through the skin resulted in primary and secondary hyperalgesia which lasted for 7 days. The dorsal incision also induced allodynia which lasted one week. The third

reason for choosing the dorsal incision model was its suitability for the von Frey filaments to evoke mechanical nociception. The dorsal incision allows the experimenter to also assess the *cutaneous trunci* muscle reflex (CTMR) in response to von Frey filaments application. The *cutaneous trunci* muscle is a broad sheet of skeletal muscle that runs rostrocaudally on the rat dorsum. Nociceptive stimuli applied to the dorsal surface results in a localized reflex contraction.

The results from this study clearly show that greater sensitivity to mechanical nociceptive stimulation was most apparent in the aged rats. Fischer 344 rats, young and aged, became more sensitive with repetitive testing. The baseline responses to repetitive mechanical stimulation were significantly higher in aged rats than young rats. In contrast to our findings in young Fischer 344 rats, earlier studies on 1.5-month old Sprague Dawley rats reported decreased sensitivity with repeated testing (Mujenda et al. 2007). In that study, primary allodynia peaked between 2 and 4 hours and returned to baseline levels by 24 hours (Mujenda et al. 2007). In our study, the young Fischer 344 rats did display some minor increased sensitivity over the testing period but it was not significant. The rat strain could be the reason for differences in sensitivity between young Sprague-Dawley and Fischer 344 rats. Fischer 344 rats have been reported to have greater sensitivity to punctate mechanical stimulation and greater allodynia/hyperalgesia after carrageenan-elicited hindpaw inflammation compared to Sprague-Dawley and Lewis rat strains (Fecho et al. 2005). Yet in a neuropathic pain study, baseline paw withdrawal thresholds to von Frey filament stimulation did not differ among Fischer 344, Lewis, Brown Norway, or Sprague-Dawley rat strains (Rode et al. 2007). In response to the spared nerve injury ligation, the Fischer 344 rats developed mechanical hyperalgesia the quickest and most severely of the rat strains. The discrepancies in response to nociceptive testing may be attributed to differences in

genetics of the strain and/or neuroendocrine responses to stress (Dhabhar and McEwen 1997; Dhabhar, McEwen, and Spencer 1993).

The results from our study indicate that age may influence the development of primary hyperalgesia. Aged rats did not develop significant primary incisional hyperalgesia as did young rats. At present time, there are no literature reports on primary hyperalgesia in aged rats. In young rats studied in our project, primary hyperalgesia occurred 3 hours after incision and lasted until POD 8. Very similar timing for primary hyperalgesia that peaked at 4 hours and remained elevated for up to 7 days was reported in young Sprague-Dawley rats (Duarte et al. 2005; Mujenda et al. 2007). The absence of hyperalgesia in aged rats may be partly due to the greater baseline sensitivity of aged rats, therefore obscuring any measurable change in cutaneous pain perception in response to the incision. This interesting age-related finding needs further investigation.

A very interesting and intriguing result from the present study is the observation that age differences in graded response depend on the intensity of the mechanical nociceptive stimulus; at lower filament forces, differences between young and aged rats were more apparent. The sensitivity to higher forces, e.g., 10 g, was similar in aged and young rats. The increased graded responses measured in the aged Fischer 344 rats may be a reflection of age-related differences in descending modulation of afferent nociceptive activity. Descending modulatory pain pathways 'dampen' the response to repetitive stimuli (Traub 1997). These descending modulatory pain pathways degenerate with age (Kitagawa et al. 2005) reducing the ability to 'dampen' the response thereby allowing greater afferent activity in the spinal cord dorsal horn. In our study, this was measured as greater sensitivity and a greater maximal graded response to filament forces in the aged rats.

The baseline graded response values in aged rats were significantly higher than in young rats but after incision, secondary hyperalgesia was absent in both aged and young rats. This is a somewhat surprising finding and difficult to understand, especially in young rats. In the modifications to the adopted experimental model of postoperative pain, the incision size was increased to 2 cm to provide larger testing area for secondary hyperalgesia. The reason for the lack of secondary hyperalgesia in response to incision in young rats is unknown. In aged rats, the factor that contributed to the absence of secondary hyperalgesia may be the increased baseline sensitivity that could interfere with the measurements performed after incision.

The results from this project are novel and very intriguing because some of them cannot, at least at this time, be fully explained due to poor understanding of and limited reports on the effects of age on postoperative pain processing especially at the spinal cord level. There is a need for ongoing study to further develop this model and study the effects of age on postoperative pain. The present findings form a basis for future investigation by providing not only new knowledge but also useful information for the experimental design of postincisional pain studies on the aged rat model.

The effect of age on postoperative pain is unknown therefore the results of this project are unique and provide an initial insight into the effects of aging on mechanism of postoperative pain at the level of the spinal cord, a critical site for pain transmission. New knowledge of this mechanism and specific age-induced changes in pain processing may lead to development of improved management of postoperative pain in the elderly. More effective postsurgical analgesia in elderly patients will result in quicker recovery, reduced immobility-induced morbidity, and reduced incidence of chronic pain development. Ultimately, the novel findings from this project and future research studies will help to better understand the age-related

changes in the complex pain syndrome process. More research is needed to help reduce the striking incidence of unsuccessful management of postoperative stress, caused by unrelieved pain. The elderly who suffer unnecessary pain face the risk of the detrimental consequences of unrelieved pain, increased morbidity and mortality.

Acknowledgments

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Figure 1. The effect of incisional surgery on force-response curves for primary allodynia and hyperalgesia at baseline, 3 hours postincision, postoperative (POD) 1 and POD 8 after incision in young (left panel) and aged (right panel) Fischer 344 rats. Data represents the mean ± S. E. of 8-9 rats (*$p < 0.05$ for control vs surgery).

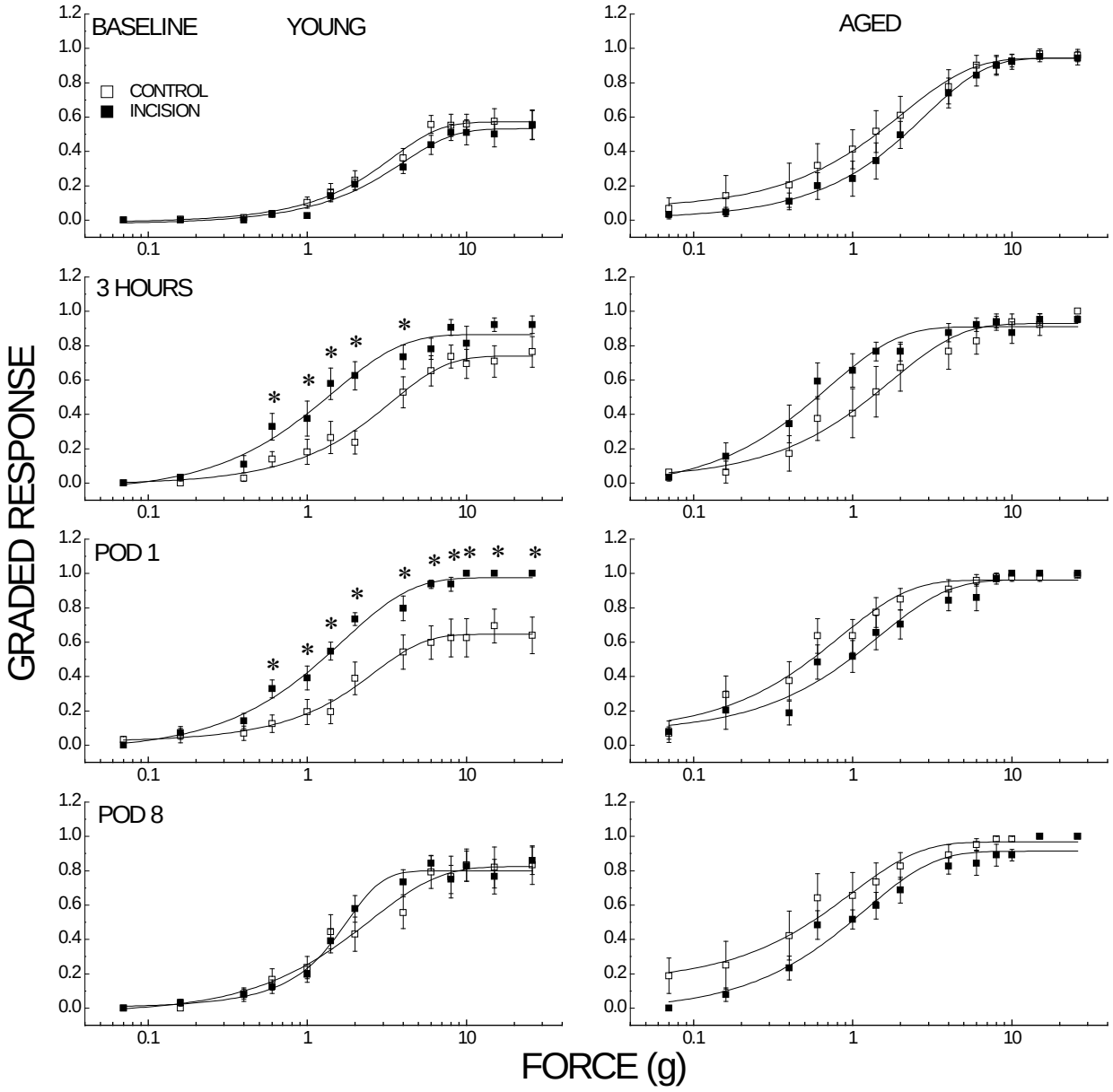
BASELINE
YOUNG
AGED
CONTROL
INCISION
3 HOURS
POD 1
POD 8
GRADED RESPONSE
FORCE (g)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0.1
1
10
*

Figure 2. Force response curves for ipsilateral secondary hyperalgesia at baseline and 6 hours postincision for young (left panel) and aged (right panel) Fischer 344 rats. Data represent the mean ± S. E. of 8-9 rats (*$p < 0.05$ for control vs incision).

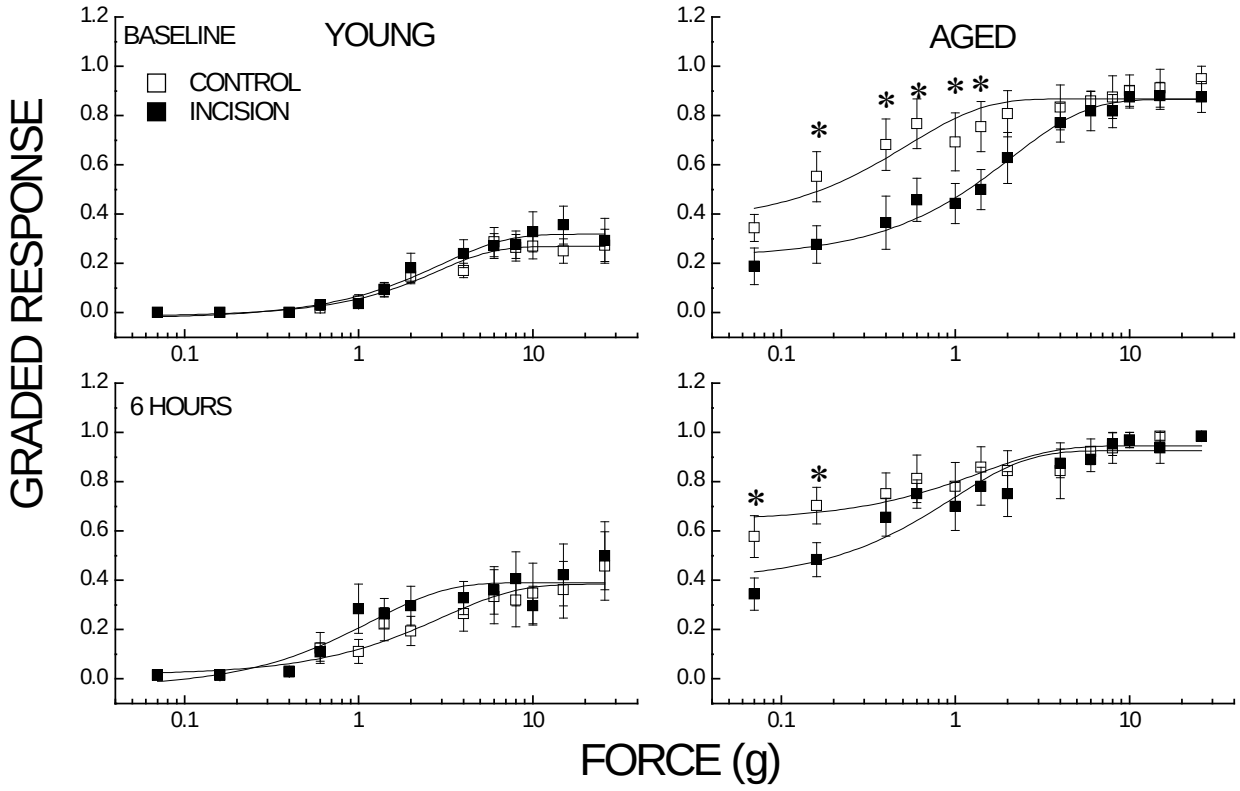
GRADED RESPONSE
BASELINE
YOUNG
CONTROL
INCISION
AGED
6 HOURS
FORCE (g)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.1
1
10
*

Figure 3. Force-response curves for contralateral secondary hyperalgesia at baseline and 6 hours postincision for young (left panel) and aged (right panel) Fischer 344 rats. Data represent the mean ± S. E. of 8-9 rats (*$p < 0.05$ for control vs incision).

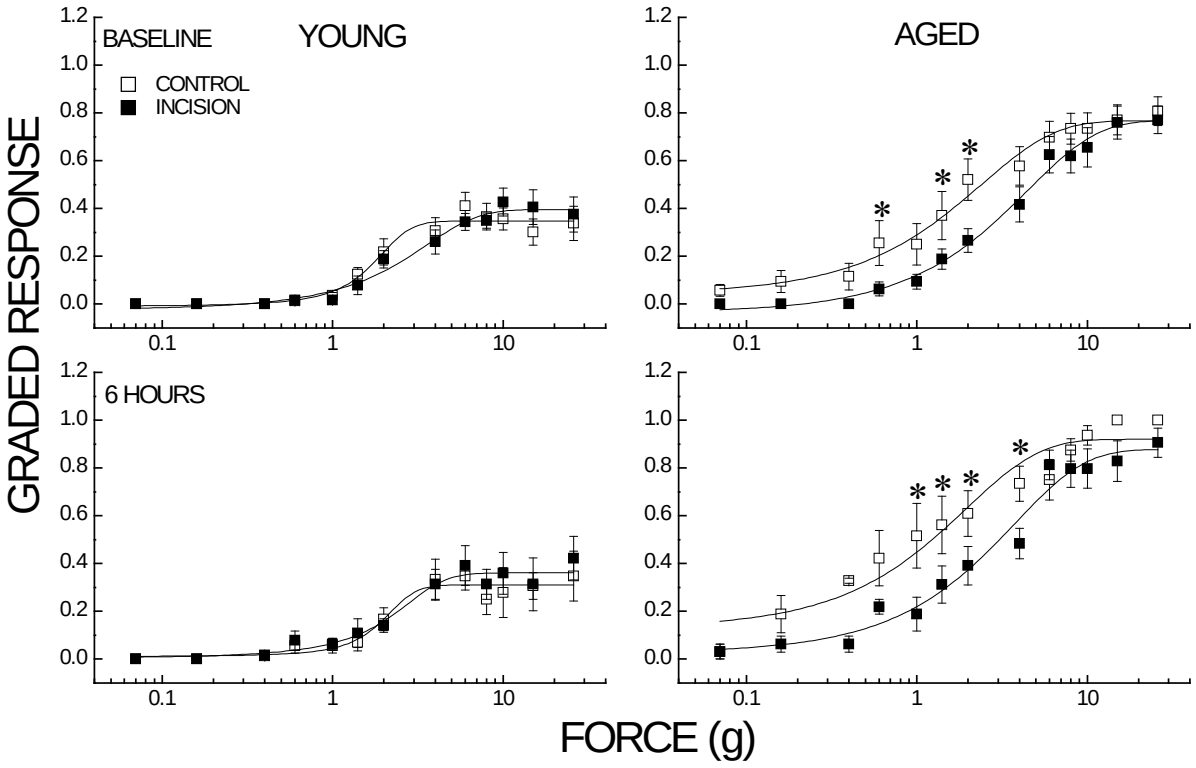
BASELINE
YOUNG
AGED
CONTROL
INCISION
6 HOURS
GRADED RESPONSE
FORCE (g)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0.1
1
10
*

Figure 4. Time course of graded response of CTMR and primary hyperalgesia to 0.6 g, 2 g, and 10 g force fibers. Testing was performed at baseline (Base), 3 hours (3h), 6 hours (6h), and on postoperative days (D) 1, 3, 6, 8, 10, and 14 for young (left panel) and aged (right panel) Fischer 344 rats. Data represent the mean ± S. E. of 8-9 rats (*$p < 0.05$ for control vs surgery).

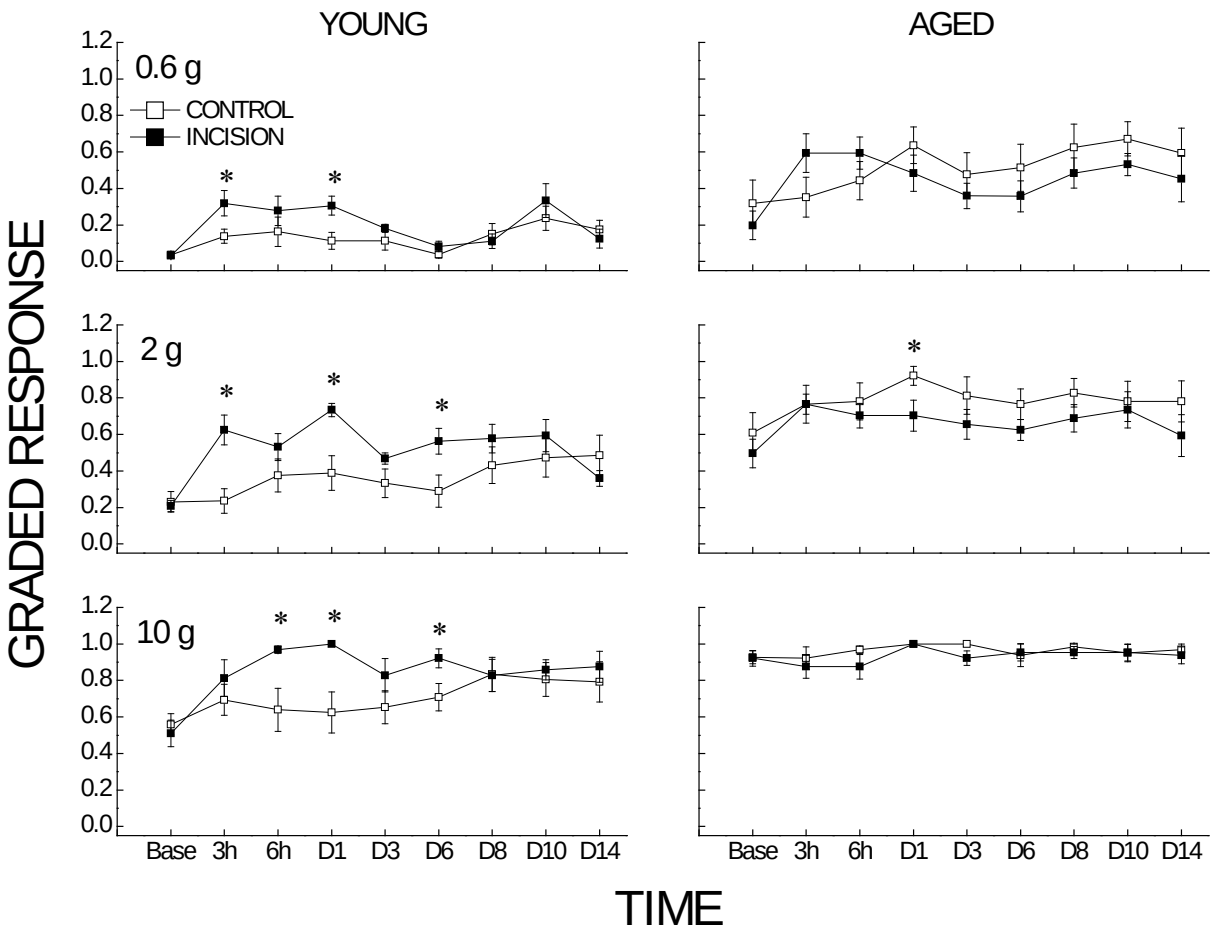
YOUNG
AGED
0.6 g
2 g
10 g
CONTROL
INCISION
GRADED RESPONSE
TIME
Base
3h
6h
D1
D3
D6
D8
D10
D14
0.0
0.2
0.4
0.6
0.8
1.0
1.2
*

Figure 5. Effect of age on primary allodynia/hyperalgesia. The results are presented for three groups of the von Frey filaments forces, low (0.07-0.6 g), mid (1-6 g), and high (8-26 g), at baseline, 3 hours postincision, postoperative day (POD) 1 and POD 8. Data represent the mean ± S. E. of 8-9 rats (*$p < 0.05$ for control vs incision; † $p < 0.05$ for young control vs aged control and/or aged incision.

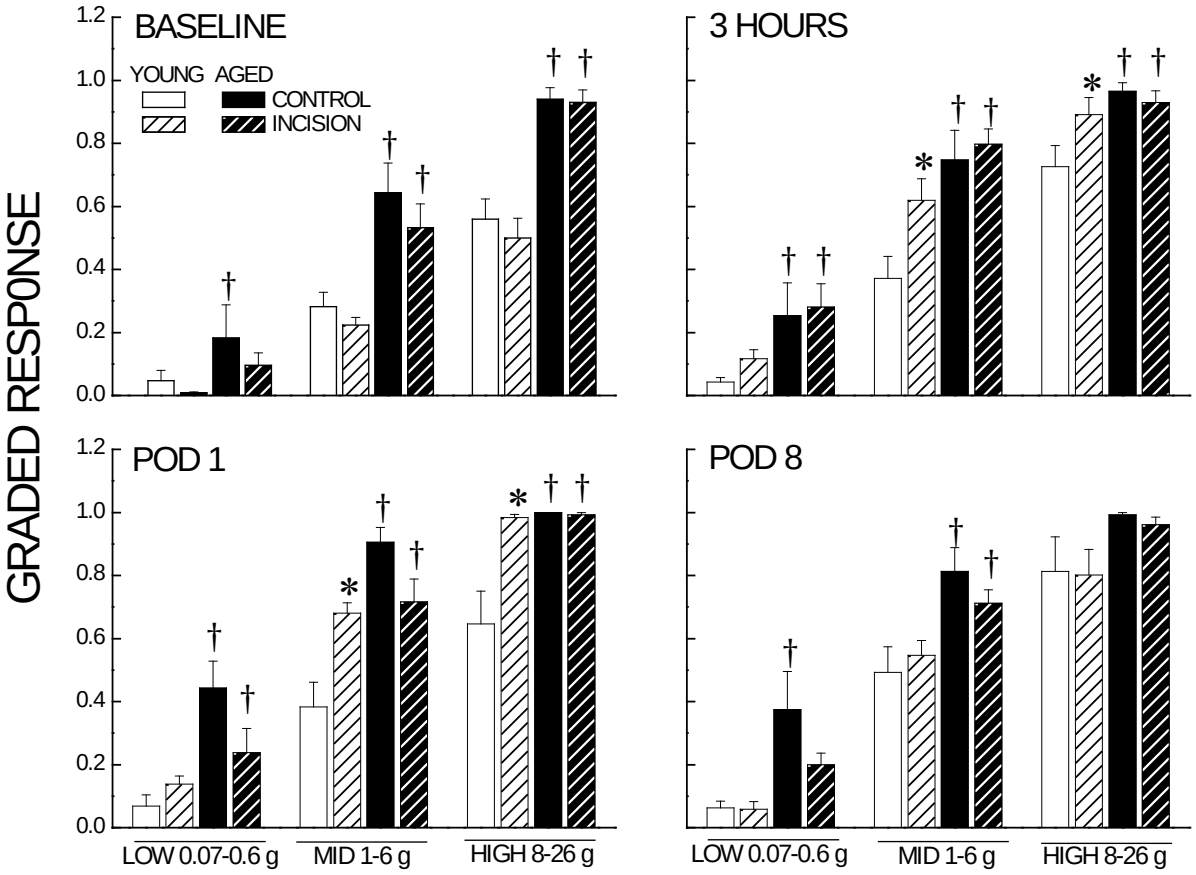
GRADED RESP0NSE
BASELINE
3 HOURS
POD 1
POD 8
YOUNG AGED
CONTROL
INCISION
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LOW 0.07-0.6 g
MID 1-6 g
HIGH 8-26 g

Fig 6. Effect of age on ipsilateral secondary hyperalgesia (left panel) and contralateral secondary hyperalgesia (right panel). The results are presented in three force groups of the von Frey filaments: low (0.07-0.6 g), mid (1-6 g), and high (8-26 g) at baseline. Data represent the mean ± S. E. of 8-9 rats († $p < 0.05$ for young control vs aged control and/or aged incision).

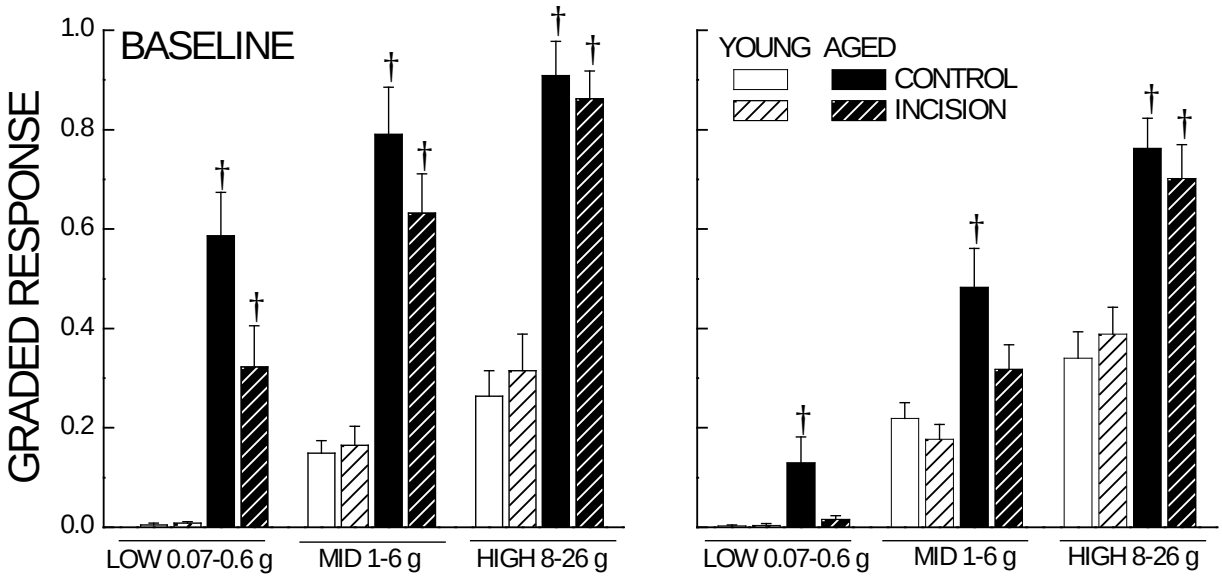
BASELINE
GRADED RESPONSE
1.0
0.8
0.6
0.4
0.2
0.0
LOW 0.07-0.6 g
MID 1-6 g
HIGH 8-26 g
YOUNG AGED
CONTROL
INCISION
LOW 0.07-0.6 g
MID 1-6 g
HIGH 8-26 g

Figure 7. The effect of age on development of sensitization to the cutaneous truncii muscle reflex graded response. Responses to 2 g von Frey filaments fibers were measured in 5 different sessions in young and aged Fischer 344 rats. Data represent the mean ± S.E. of 17 young and 16 aged rats (*$p \leq 0.05$ for young vs aged).

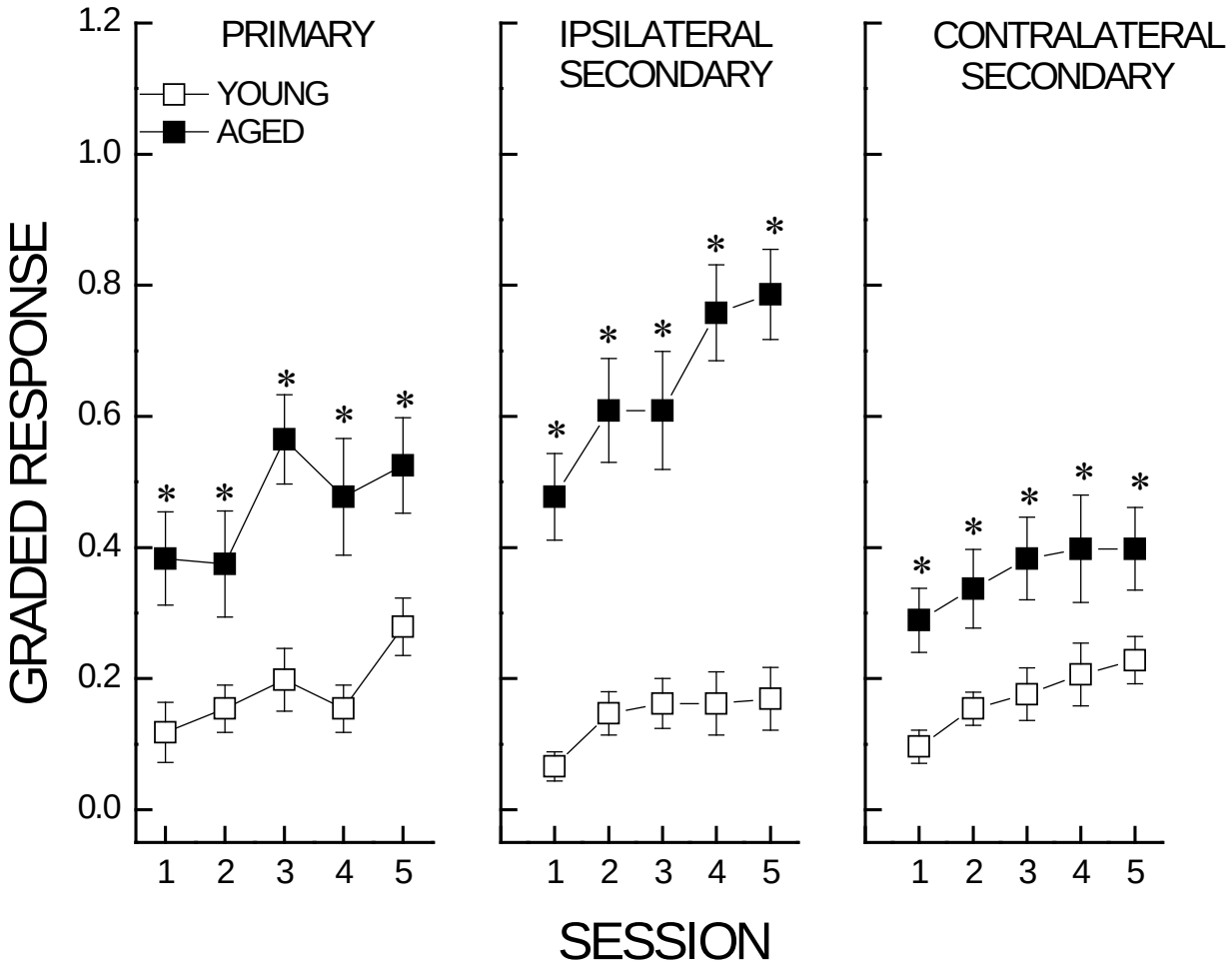
PRIMARY
IPSILATERAL SECONDARY
CONTRALATERAL SECONDARY
YOUNG
AGED
GRADED RESPONSE
SESSION
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1
2
3
4
5
*

Table 1. Comparison of graded responses between Sprague-Dawley rats and two ages of Fischer 344 rats.

	SD 1.5 - months	F344 5-7 months	F344 22-23 months
	18 mN (≈2 g)	2 g	2 g
Baseline	0	0.208	0.496
3 hours	0.38	0.625	0.766
POD 6	0.19	0.563	0.625
	90 mN (≈10 g)	10 g	10 g
Baseline	0.55	0.559	0.922
3 hours	0.88	0.813	0.875
POD 6	0.71	0.922	0.953

Notes: Filament forces of 2 g (≈ 18 mN) and 10 g (≈ 90 mN) (from: Touch Test™ Sensory Evaluators, Semmes Weinstein von Frey Aesthesiometers, Stoelting Co, Wood Dale, IL). Data for the Sprague-Dawley rats were obtained from Duarte, et al. (Duarte et al. 2005). SD, Sprague-Dawley; F344, Fischer 344; 3 hours, 3 hours postincision; POD 6, postoperative day 6.

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TRANSITION REMARKS

In Chapter III, an experimental model of postoperative pain in aged rats was presented, and the effects of age on incisional acute pain were studied. Although the young rats developed allodynia lasting through postoperative day 3 and primary hyperalgesia lasting until postoperative day 8, the aged rats did not develop primary hyperalgesia. Furthermore, neither young nor aged rats developed secondary hyperalgesia. Throughout the course of the experiments, there was consistently greater baseline sensitivity and maximal graded response to nociceptive mechanical stimulation in the aged rats. As this phenomenon had not been previously reported, we sought to discover a possible mechanism for the difference in sensitivity between young and aged rats.

Neuroplasticity of the spinal cord, in particular, central sensitization, involves an intricate interplay of neuronal excitation, neurotransmitter release, postsynaptic membrane responses, and eventual changes in protein expression. The postsynaptic N-methyl-D-aspartate (NMDA) receptor is integral for the initiation and maintenance of neuroplasticity in the spinal cord dorsal horn. Expression of the NMDA receptor fluctuates in response to nociceptive stimuli. The NR1 subunit is ubiquitous and is requisite for a functional receptor. The subsequent studies were designed to confirm the findings from the studies presented in Chapter II and analyze changes in the NR1 expression in spinal cord in response to nociceptive stimulation in aged and young Fisher 344 rats.

AGE-RELATED CHANGES IN SENSITIVITY TO NOCICEPTIVE STIMULI AND EXPRESSION OF SPINAL CORD NR1 IN FISCHER 344 RATS

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CHAPTER IV

AGE-RELATED CHANGES IN SENSITIVITY TO NOCICEPTIVE STIMULI AND EXPRESSION OF SPINAL CORD NR1 IN FISCHER 344 RATS

Abstract

The purpose of this study was to explore the mechanism underlying the age-related increase in sensitivity and sensitization to nociceptive stimulation. Young or aged male Fischer 344 rats were exposed to von Frey filaments (0.07g – 26g), applied sequentially to primary, secondary, and tertiary regions of dorsal skin and western analysis was used to measure expression of NR1 in spinal cord. Aged rats had significantly greater responses to nociceptive stimulation than young rats; this age difference was more pronounced at higher filament forces. Nociceptive stimulation increased spinal NR1 expression but this increase was statistically significant only in young rats. A significant negative correlation between spinal NR1 expression and responses to mechanical stimulation was observed in aged rats. Aged rats demonstrate greater sensitivity and sensitization to repeated nociceptive stimulation than young rats.

Key words: age – nociception – NR1 – pain

Introduction

Age-related differences in pain perception have been reported to increase (Pickering et al. 2002), decrease (Chakour et al. 1996), or not change (Aubrun et al. 2003; Aubrun et al. 2002; Chakour et al. 1996). Many factors may impact the outcomes of pain studies, e.g. stimulus type and size (Chakour et al. 1996; Pickering et al. 2002; Washington, Gibson, and Helme 2000), gender (Aubrun et al. 2005; Pickering et al. 2002), experimental method used to assess pain intensity (Gagliese and Katz 2003; Gagliese et al. 2005) or affective state at time of testing (Chakour et al. 1996; Pickering et al. 2002; Washington, Gibson, and Helme 2000). When studied at the level of neural trafficking in afferent nociceptive inputs for the spinal cord, reasons for apparent disparities in human studies can be clarified.

Afferent nociceptive inputs activate descending modulatory pathways which facilitate nociception after injury but convert to predominantly inhibitory modulation with continuous noxious input (Herrero and Cervero 1996; Ren and Dubner 1996). These endogenous pathways are very effective in the young adult in reducing pain experienced upon subsequent exposure to a painful stimulus (Washington, Gibson, and Helme 2000) but are subject to degeneration with age. In the aged adult, the less effective descending pathways allow for greater afferent traffic into the central nervous system (Edwards, Fillingim, and Ness 2003; Pickering et al. 2002; Washington, Gibson, and Helme 2000). Such a phenomenon is also present in aged rats, based on the observation that blockade of descending inhibitory pathways results in little increase in afferent activity, whereas a dramatic increase occurs in young rats following a thermal noxious stimulus (Iwata et al. 2002).

Peripheral activation of nociceptors induces release of excitatory neurotransmitters, e.g. substance P and glutamate, in the dorsal horn of the spinal cord. Glutamate activates n-methyl-

D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and kainate receptors on postsynaptic neurons which are integral to nociceptive transmission (De Biasi and Rustioni 1988; Dougherty et al. 1992). If the afferent input is of sufficient magnitude, NMDA receptors can be activated which increases the overall response to the nociceptive inputs (Chen and Huang 1992). Activation of the NDMA receptor is important in the initiation and maintenance of central sensitization whereby nociceptive activity is enhanced (Liu and Sandkuhler 1998).

Age-associated decreases in neurotransmitters (e.g., serotonin, norepinephrine, glutamate, gamma-amino-butyric acid, glycine) (Amenta, Zaccheo, and Collier 1991; Ko et al. 1997; Spokes 1979; Virgili et al. 2001), and neurons (Iwata et al. 2002) within the nociceptive pathways may result in altered pain perception. In neuropathic pain, NR1 subunit expression in the spinal cord increased (Gu et al. 2008; Roh et al. 2008), whereas in inflammatory (Caudle et al. 2005; Zou, Lin, and Willis 2002) or noxious heat pain (Brenner et al. 2004), no increase was observed. In aged Wistar rats spinal cord NR1 expression is decreased (Monti, Virgili, and Contestabile 2004). Spinal cord NR1 subunit expression in aged Fischer 344 rats has not been reported.

Studies completed recently in our laboratory on the effect of age on postincisional pain in Fischer 344 rats, showed that aged rats exhibited greater sensitivity to nociceptive mechanical stimulation than young rats (cf. Chapter II). With repetitive nociceptive testing sessions, both young and aged rats exhibited sensitization. The present studies were designed to confirm previous observations of differences in sensitivity and sensitization and to determine the involvement of NR1 expression in these effects. We hypothesized that spinal cord NR1

expression will increase in response to nociceptive stimulation and that age-related differences in magnitude of NR1 expression will be evident.

Methods

Animals

Male Fisher 344 rats aged 4-6 months (young, n=34) and 19-21 months (aged, n=31) were obtained from the National Institute on Aging and housed in pairs in clear polycarbonate cages in the University of North Texas Health Science Center vivarium. The rats were maintained at 22-24°C, under a 12-hour light/dark cycle, with lights on at 0700 h. Rats were given *ad libitum* access to food and water. After the last testing period, rats were euthanized by decapitation and spinal cords were harvested. All procedures involving the rats were approved by the Institutional Animal Care and Use Committee of the University of North Texas Health Science Center at Fort Worth.

All experimental rats were subjected to nociceptive testing procedures adopted from Duarte et al., (2005) and Mujenda et al. (2007), with modifications. Rats were handled during five 5-min sessions over three days to acclimate them to the experimenter and testing environment. During these sessions the rats learned to remain still on the experimenter's forearm with nose tucked into the crook of the arm.

The day before the first testing, the specific dorsal skin area was prepared by clipping the fur and marking specific testing areas with indelible markers. To facilitate the preparation, rats were anesthetized with isoflurane at 5% until cessation of movement then continued with 2-3% isoflurane via nose cone. Heart rate and oxygen saturation were monitored with pulse oximetry. During the test area preparation and recovery the rats were placed on a heated plate (39°C)

covered with a soft, absorbent pad. The primary test region was marked parallel to the vertebral column 1 cm to the right beginning at the iliac crest and extending rostrally 2 cm. The secondary (right of vertebral column) and tertiary (left of vertebral column) test regions were marked 2 cm from the primary region site avoiding bony prominences (Figure 1). These marked testing sites were used for three testing sessions. Control rats were subjected to all procedures as the test group but did not undergo von Frey filament testing. Rats were housed in pairs until day of session 1 testing then housed singly.

Testing of sensitivity to von Frey filaments

Cutaneous trunci muscle reflex (CTMR) (Theriault and Diamond 1988) to von Frey filament stimulation was recorded. Thirteen (13) filaments (force from 0.07 g to 26 g) were tested sequentially beginning with the area marked as the primary region followed by testing on the secondary region (2 cm from primary, ipsilateral), and then followed with testing the tertiary region (2 cm from primary, contralateral). Each filament was briefly pressed to the skin until the filament bent and then it was removed. Filaments were applied four times at 2-3 second intervals. CTMR graded responses were scaled as 0, 0.5, or 1 for no reflex, a small reflex, or large reflex, respectively, as previously described (Duarte et al. 2005; Mujenda et al. 2007). For each testing session, the four responses to a filament were averaged and recorded as the response to the filament. Three test sessions were conducted over two days. Session 1 and session 2 results are reported.

Age-related differences in response to nociceptive testing by von Frey filaments were expressed as force-response curves by fitting the mean CTMR graded response to a 4-parameter logistic dose response function of filament force. Comparisons in maximal responses and

effective force 50 (EF50) were derived from the resulting curves and used to describe differences in sensitivity to nociceptive testing. EF50 was measured at the mid-point of the sigmoid curves between 0 (no graded response) and the maximal response elicited for the age group and region tested. An increase in sensitivity, or responsiveness to nociceptive stimuli, is evident as a leftward shift of EF50. An increase in sensitization, or an increased responsiveness upon repeated exposure to nociceptive stimuli, is evident as an increase in graded response from session 1 to session 2.

The von Frey filaments were divided into three groups based on the shape of sigmoidal force-response relationships obtained in young rats in previous studies (cf. Chapter II). Briefly, the lowest force filaments from 0.07 to 0.6 g corresponded to the flat portion (intercept) of the curve. The linear part of the curve corresponded to the mid-range filament forces, 1-6 g. The plateau part of the force-response curve corresponded to the high force filaments from 8 g to 26 g.

Tissue processing and western blot analysis

Harvested spinal cords (thoracic level 10 to lumbar level 1, L_1) were equally sectioned into fourths, washed with PBS and immersed in lysis buffer containing protease and phosphatase inhibitors as described previously (Singh et al. 1999). After homogenization of the L_1 section, samples were centrifuged at 99,000 x *g* for 15 min at 4°C and the resulting supernatants were evaluated for total protein concentrations using the Bio-Rad DC (Bio-Rad Labs, Hercules, CA) protein assay kit (based on the method of Lowry, *et al*. (1951). L8 cell line lysate (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and rat brain microsomal preparation (Millipore, Temecula, CA) served as negative and positive methodological controls, respectively. Sample

lysates (100 µg protein/lane), L8 lysate (20 µg protein/lane) and rat brain microsomal preparation (40 µg protein/lane) were loaded onto a 7.5% SDS-PAGE, subjected to electrophoresis, and subsequently transferred onto a polyvinylidene difluoride membrane (PVDF; 0.22 µm pore size; Bio-Rad). The membrane was blocked overnight with a 3% BSA in 0.2% Tween-containing Tris-buffered saline (TBS-T) solution before application of the primary antibody. The following primary antibodies were used: for the detection of NR1, mouse anti-NR1 (1:1400, Millipore, Temecula, CA) and for detection of actin, mouse anti-actin pan Ab-5 (1:2000, Thermo Fisher Scientific). Antibody binding to the membrane was detected using a secondary antibody, goat anti-mouse, conjugated to horseradish peroxidase (NR1, 1:12,5000; actin 1:5000, Pierce, Rockford, IL) and was visualized using enzyme-linked chemiluminescence (SuperSignal West Pico, Pierce) with the aid of the UVP (Upland, CA) imaging system.

Statistical analysis of data

Data are presented as the mean ± S. E. for each filament for separate groups of 15-17 rats. To determine the effects of age on graded response, data were analyzed by 4-way ANOVA with Age as a between group factor and Session, Region, and Force as within-group factors. To determine the effect of age on graded response to categories of filaments (low, mid, and high), data were analyzed by two-way ANOVA with Age and filament group Force as between group factors separately for the primary, secondary and tertiary regions. The effect of age and nociceptive testing on NR1 expression was determined by two-way ANOVA with Age and Test as between group factors. Planned individual comparisons between Age and Test were performed using single degree-of-freedom F tests within the two-way interaction for each set of data.

Data sets were subjected to nonparametric statistics for comparison to parametric statistic results. Data are presented as the median with 5^{th}, 25^{th}, 75^{th}, and 95^{th} percentiles. To determine the effects of age and nociceptive testing on NR1 expression, planned individual comparisons between Age and Test were performed using Kruskal-Wallis one-way ANOVA.

Lastly, Pearson correlation coefficients were calculated to determine the degree of linear relation between intensity of graded response and NR1 expression of young and aged rats for sessions 1 and 2 in each filament force group (low, mid, high) and by region (primary, secondary, and tertiary). Individual Pearson correlation coefficients and *p* values are reported in Table 1. Statistical significance was defined as $p < 0.05$.

Results

Effect of age on sensitivity to von Frey filament nociceptive testing

The effect of age on the CTMR graded response to von Frey filaments (0.07-26 g) in young and aged rats is presented in Figure 2. The maximal graded response to von Frey filament stimulation in aged rats was two times greater than the maximal graded response in young rats in session 1 for all three regions (Figure 2, left panel). The EF50 was also greater in the young rats during session 1 for all three regions. No significant differences between age groups were evident for lower forces (0.07, 0.16, 0.4, and 0.6 g) in the primary, secondary, and tertiary regions, whereas significant differences were detected in responses to mid force filaments in the primary (2, 4, and 6 g, $p < 0.006$), secondary (1, 2, 4, and 6 g, $p < 0.036$), and tertiary regions (2, 4, and 6 g, $p = 0.001 – 0.049$). For all high force fibers (8, 10, 15, and 26 g), significant differences were detected in the primary, secondary, and tertiary regions ($p < 0.002$).

The graded responses increased in both age groups in all three regions in session 2 compared to session 1 (Figure 2, right panel). The difference in maximal graded response between age groups widened to a three-fold difference in the secondary and tertiary regions. In session 2, no significant differences in graded response were measured between age groups for low forces (0.07-0.6 g) in any of the three regions. In the primary region, no significant differences between young and aged were present for mid-force fibers (1-6 g). In the secondary ($p < 0.042$) and tertiary regions ($p < 0.006$), mid force fibers 1.4, 2, 4, and 6 g evoked significantly greater responses in the aged rats, as did the high force (8-26 g) fibers in all three regions ($p < 0.003$). In aged rats, with the increase in maximal responses, the EF50 shifted right-ward (less sensitive) in all three regions. From session 1 to session 2, in young rats, the EF50 shifted right-ward in the primary region, the area of greatest increase in maximal response; there was no change in the secondary region, and a leftward shift in the tertiary region. An interesting phenomenon in regional differences between age groups became apparent when comparing maximal graded responses. The graded maximal response in young rats increased from session 1 to session 2 by 75-82%. In aged rats, maximal graded response increased by 22% in the primary region, 69% in the secondary region and 140% in the tertiary region.

The effect of age on the graded response to low, mid, and high category of von Frey filament forces are summarized in Figure 3. When the summary dataset was analyzed, no significant differences between young and aged were detected in the low force filament group in any of the three test regions for sessions 1 and 2 (Figure 3, left panel). In both mid ($p < 0.004$) and high ($p < 0.001$) force category, aged rats had significantly greater graded responses in all three regions for both sessions with the exception of the mid force category, primary region, session 2 ($p = 0.221$, Figure 3, middle and right panels).

Comparison of the graded responses within each age group and between sessions showed that in young rats, graded responses in all three regions increased from session 1 to session 2 by 54-73% in the mid force and by 64-91% in the high force category. In contrast, in aged rats, there was no increase in graded response from session 1 to session 2 in the primary region for mid force and only 12% increase in the high force category. However, aged rats showed increased graded response from session 1 to session 2 in the secondary (52% for mid and 62% for high force) and tertiary regions (109% for mid and 125% for high force).

The 4-way ANOVA indicated an interaction among Age, Session, and Region. Increases in graded response from session 1 to session 2 were evident in young and aged rats except for the primary region where aged rats did not have an increase in graded response between sessions. Age effects on region were evident in the magnitude of graded response for the secondary region which was approximately three-fold greater in aged rats than young rats.

The effect of age and von Frey filament nociceptive stimulation on spinal NR1 expression

The NR1 expression in L_1 spinal cord segments in response to nociceptive stimuli is shown in Figure 4 for young and aged rats. No significant differences in NR1 expression were evident between young and aged controls ($p = 0.127$). Young rats exposed to nociceptive testing had a significantly higher level of NR1 expression when compared to young control rats not exposed to the testing ($p = 0.046$). In aged rats exposed to nociceptive testing, there was greater NR1 expression when compared to young controls, however, the increase was not statistically significant ($p = 0.076$).

Whereas the only significant difference in means among the groups was between young control and young tested, nonparametric analysis of the densitometric data revealed additional

group differences as well as age-related shifts in distribution of individual data (Figure 5). As in the mean differences, there was also a statistically significant difference in the medians between young control and young tested ($p = 0.024$). Additionally, the aged control rats had a significantly greater median compared to the young control group ($p = 0.033$). Aged tested rats had a greater median than aged control but the difference was not statistically significant ($p = 0.078$). Additionally, there was a notable upward shift in the distribution of data within the range above the median (50-75th percentile) in the aged tested rats.

Correlation between sensitivity to von Frey filaments and NR1 expression.

A Pearson correlation analysis was applied to assess the extent to which NR1 expression of individual rats was quantitatively related to their degree of reactivity to low, mid, or high force von Frey stimulation (Table 1). Graded responses to the low, mid, and high force filaments were indeed correlated with relative intensity of NR1 expression, though the magnitude and direction of this relationship was age-dependent. Significant correlations (Pearson $r > 0.5$ and $p < 0.05$) were present for 9 of 18 region/sessions in the aged rats (Table 1). In young rats, NR1 expression showed no significant relationship with graded response, although a significant positive correlation was present for the secondary region during session 2, following stimulation with high force filaments ($r = 0.561$, $p = 0.019$). In contrast, among the aged rats, there was a consistently negative relationship between NR1 expression and graded response during both sessions, for the mid and high force filament categories. As suggested in scatter plots from session 2, (Figure 6), the negative correlations among the old rats were driven by the relatively greater filament sensitivity for rats with NR1 intensities below 0.10.

Discussion

The results from this study showed that significantly increased sensitivity to mechanical nociceptive stimulation in aged rats was negatively correlated to the expression of NR1 in spinal cord. This novel finding suggests involvement of the spinal NMDA system in the mechanism associated with age-related changes in pain perception. Differences in sensitivity to von Frey filament stimulation were significant between young and aged rats. Initial responses were lower in young rats than aged rats but this response by young rats increased 75-82% between session 1 and session 2. Aged tested rats had higher initial graded responses than young rats but the responses did not increase much with the subsequent testing session. These age differences were most evident with mid (1–6 g) and high (8–26 g) forces of filament stimulation. Rats exposed to nociceptive stimulation had increased expression of NR1 in spinal cord, but this increase was statistically significant only in young rats. A greater graded response was positively correlated with increased NR1 expression in young rats but only in session 2, secondary region, for the high force filaments. In aged rats, this correlation was remarkably opposite showing a negative correlation with graded response intensity and NR1 expression. The negative correlation was present in 9 of 18 region/session and filament force categories.

Von Frey filaments are commonly used to evaluate pain in rat models of neuropathic, inflammatory, and postoperative pain. Changes in pain perception in response to pain testing modalities are often reported as change from baseline. Whereas a greater baseline sensitivity in aged rats to von Frey filament stimulation has indeed been reported previously (Kitagawa et al. 2005), it does not seem to be the case that a positive shift in pain sensitivity is universal, as an absence of baseline disparity has also been reported (Crisp et al. 2003; Cruce et al. 2001). The specific site of the von Frey testing represents an obvious source of divergent results in the

literature. In the current study CTMR was elicited by stimulating hairy skin of the back whereas the glabrous skin of the rat hindpaw has been the site of testing in the aforementioned studies (Crisp et al. 2003; Cruce et al. 2001; Kitagawa et al. 2005). The rat hindpaw has a higher threshold for withdrawal than what was required to elicit the CTMR in both young and aged rats in the current studies. Therefore it seems reasonable to speculate that the CTMR may afford a more sensitive measure of age differences in pain.

In our study, aged rats exhibited greater sensitivity and maximal graded responses than young rats. In both groups, the maximal response increased from session 1 to session 2. However, the young rats were fairly consistent with a 75-82% increase in the three areas. The aged rats demonstrated regional differences with a small increase in the primary region, a moderate increase in the secondary region, and large increase in the tertiary region. A similar pattern was noted in the histogram data (Figure 3). In young rats, from session 1 to session 2 in all three regions in both mid and high forces, graded responses increased by 54-91%. For the aged rats, the histogram data resembles the force-response curve. In the primary region, the aged rats had essentially insignificant increase from session 1 to session 2 demonstrating a 'ceiling effect.' In the secondary region, a moderate increase from session 1 to session was measured (52-62%) but in the tertiary region, the greatest increase was observed (109-125%).

Nociceptive neurons of aged rats show greater spontaneous activity, more afterdischarges, and a diminished increase in electrical activity after an inflammatory agent is injected into the hindpaw (Kitagawa et al. 2005). This higher basal afferent activity does not allow for an increased response to inflammation or injury. The greater activity in aged neurons is reflected in the increased sensitivity and maximal graded response observed in aged rats reported in this study. These results are similar to findings from human studies where pain

threshold and tolerance to pressure was lower in elderly than young men, implying degeneration of descending modulatory pain pathways (Pickering et al. 2002).

Age-related greater neuronal activity (Kitagawa et al. 2005), greater sensitivity and maximal graded response, and lower pressure pain threshold (Pickering et al. 2002) may be due to degeneration of descending modulatory pain pathways from the periaqueductal gray and rostral ventral medulla. Reduced descending inhibition of nociceptive afferent inputs may result in a more pronounced graded response in the aged rat.

Additional factors may contribute to the greater sensitivity and sensitization to repeated mechanical nociceptive stimulation in the aged rats. Degeneration of myelinated A-δ fibers (Bergman and Ulfhake 2002; Sato, Sato, and Suzuki 1985) results in loss of mechanoreceptive input to the central nervous system and a resultant reduction in discriminative sensation. Unmyelinated fibers show minimal degeneration with age (Bergman and Ulfhake 2002; Verdu, Buti, and Navarro 1996) leaving C-fiber afferent nociception intact. Age-associated alteration in neurotransmitter release and/or receptor activation in the spinal cord dorsal horn may impact neuronal function. Reductions of excitatory (serotonin, glutamate, aspartate) and inhibitory (GABA, glycine) neurotransmitters occur with age in the spinal cord dorsal horn (Ko et al. 1997; Virgili et al. 2001). Combined, these factors may contribute to altered sensory function in the aged animal.

The NR1 subunit is common to all heterotetrameric configurations of NMDA-sensitive channels, and thus the current results should be reflective of overall shifts in spinal NMDAR expression No significant differences were measured in spinal cord NR1 expression between young (4-6 month old) and aged (19-21 months old) rats with parametric statistical analysis, although non-parametric analysis suggested an age-related increase. The results for spinal cord

NR1 in the current studies contrast with reports for age-related changes in spinal NR1 NMDAR subunit expression, which was lower in aged (30 month old) male Wistar rats than young (4 month old) (Monti, Virgili, and Contestabile 2004). Strain and/or age differences may be the reason for this discrepancy between the studies.

Compared to young control rats, NR1 expression increased significantly in young rats subjected to nociceptive testing. Testing aged rats also increased NR1 expression in response to nociceptive stimuli, but the increase was not statistically significant. Repetitive testing sessions is integral to many pain models. In our studies, no other nociceptive test was inflicted, i.e. capsaicin, neuropathic injury, thermal, or electrical, yet NR1 expression increased significantly with repetitive testing. Assessment of what has been believed to be a relatively innocuous method of pain testing may need further evaluation to determine the impact of von Frey filament application on experimental results.

Activation of the NMDA receptor is crucial to initiation and maintenance of central sensitization within the spinal cord dorsal horn (Woolf and Thompson 1991). NMDA receptor NR1 subunit expression has been reported to increase in response to neuropathic injury (Gu et al. 2008; Roh et al. 2008) but not increase with inflammatory (Caudle et al. 2005; Zou, Lin, and Willis 2002) or noxious heat pain (Brenner et al. 2004). Increase in NR1 expression in the spinal cord correlated with increased paw withdrawal frequency to von Frey filament stimulation (Roh et al. 2008). In our study, the young rats showed an increase in NR1 expression correlated with an increase in graded response. This positive correlation was present for only one of 18 region/session/force periods in the analysis. The von Frey filament nociceptive stimulus may be insufficient to elicit adequate increase in NR1 expression to have more region/session/force periods positively correlated with graded response. Interestingly, the aged rats had strong

negative correlations between decreasing NR1 expression and a greater graded response. This association was present in 9 of 18 region/session/force periods that were analyzed. The descending modulatory pain pathways can be facilitatory or inhibitory. Both facilitation and inhibition of spinal cord dorsal horn neuronal activity occurs simultaneously with one or the other effect predominant.

Results from this study suggest a lack of age-related differences on central sensitization. An increase in sensitization, or increase in responsiveness upon repeated exposure to nociceptive stimuli, is evident as an increase in graded response from session 1 to session 2. Young rats consistently increased graded response from session 1 to session 2 in all three regions for both mid and high forces. In aged rats, there was also increased graded response from session 1 to session 2 but only in the secondary and tertiary regions for mid and high forces. Both age groups increased graded response with repetitive nociceptive testing. This suggests that age did not have a significant effect on the neuroplasticity of the spinal cord neurons to increase responsiveness to nociceptive stimuli.

Low levels of glutamate at the rostral ventral medulla (RVM), low stimulation intensities (Zhuo and Gebhart 1992, 1997), and early in the complete Freund's adjuvant inflammation injury process (Terayama, Dubner, and Ren 2002) are associated with facilitation of the descending pathways. Higher levels of glutamate, high stimulation intensities (Zhuo and Gebhart 1992, 1997), and a delay after initial inflammatory injury (Terayama, Dubner, and Ren 2002) are characteristics associated with predominantly inhibitory effects from the RVM descending pathways. Most studies of descending modulatory pathways are conducted on young adult rats and no reports are available on age-related changes in the facilitatory component of the descending modulatory pain pathways. Although speculative, the degeneration of endogenous

pathways may affect the inhibitory arm leaving the facilitatory effects to predominate. Descending facilitatory activity may contribute to the paradoxical negative correlation between NR1 expression and sensitivity to von Frey stimulation observed for the aged rats.

In summary, results from the current studies demonstrate that aged rats had markedly greater baseline sensitivity to von Frey filament stimulation and greater maximal graded responses with regional differences than young rats. In young rats, the baseline sensitivity was lower than in aged but with repetitive stimulation, the maximal graded response to nociceptive stimulation significantly increased in all tested regions. Nociceptive stimulation resulted in increase in spinal NR1 expression in young rats and was positively correlated with the magnitude of the graded response. In aged rats, decreased NR1 expression negatively correlated with graded response. These results imply that age-related changes may be evoked by a mechanism involving the NMDA system.

Acknowledgments

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Figure 1. Diagram of testing regions. Center vertical line, vertebral column; horizontal line, level of iliac crest; solid bold line right-of-center, primary region; dashed line, right side, secondary region; dotted line, left side, tertiary region.

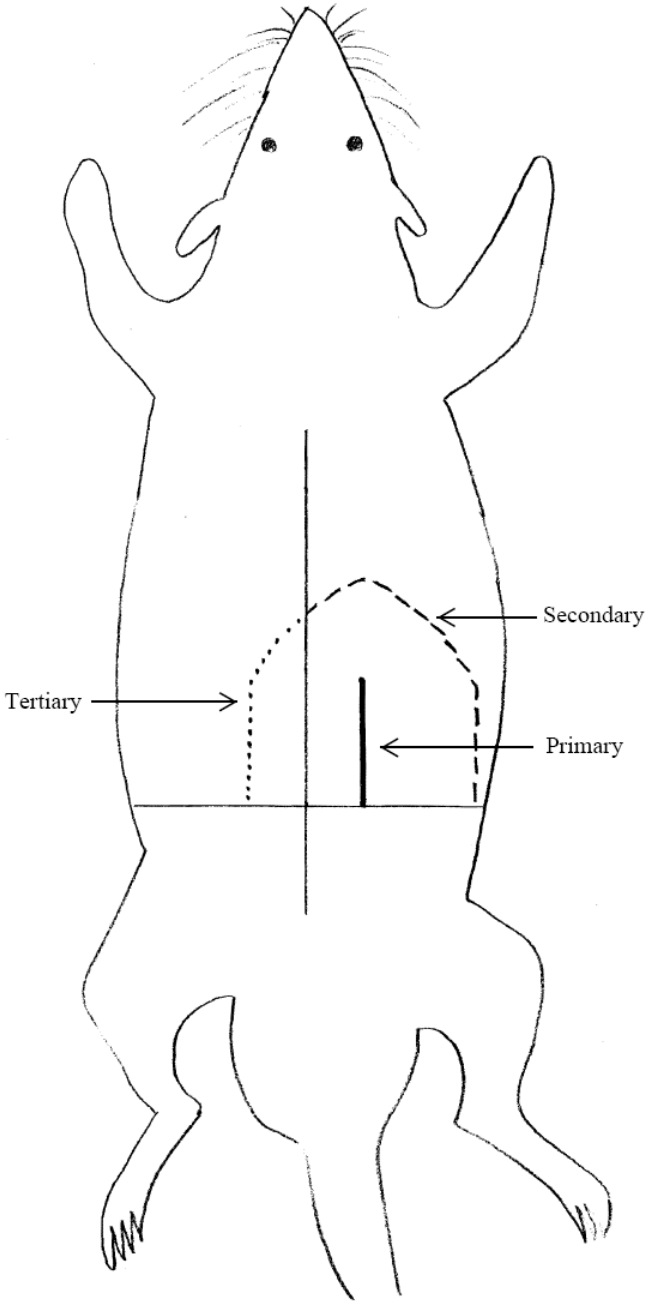

Secondary
Tertiary
Primary

Figure 2. The effect of age on graded response to von Frey filament stimulation in Fischer 344 rats. Left column, session 1 and right column, session 2, young vs aged in primary, secondary, and tertiary test regions. Data are presented as the mean ± S. E. of 16-17 rats (*$p < 0.05$ young vs aged). Abbreviations: Y, young; A, aged; MR, maximal response; EF50, effective force 50%.

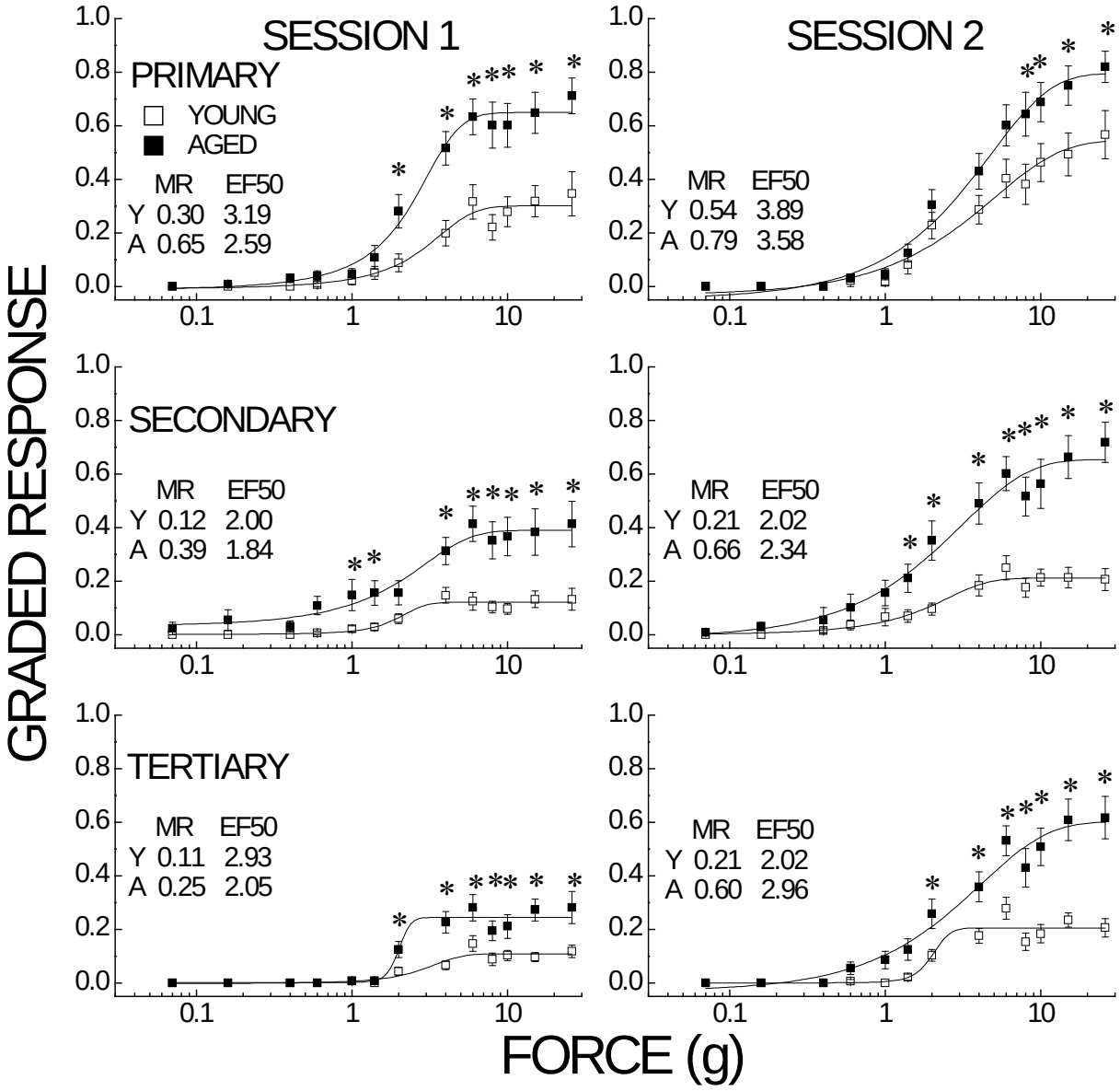

SESSION 1
SESSION 2
PRIMARY
SECONDARY
TERTIARY
YOUNG
AGED
MR EF50
Y 0.30 3.19
A 0.65 2.59
MR EF50
Y 0.54 3.89
A 0.79 3.58
MR EF50
Y 0.12 2.00
A 0.39 1.84
MR EF50
Y 0.21 2.02
A 0.66 2.34
MR EF50
Y 0.11 2.93
A 0.25 2.05
MR EF50
Y 0.21 2.02
A 0.60 2.96
GRADED RESPONSE
FORCE (g)
0.0
0.2
0.4
0.6
0.8
1.0
0.1
1
10

Figure 3. Effect of age on graded response to von Frey filament stimulation in Fischer 344 rats. The responses are to low (0.07-0.6 g), mid (1-6 g), and high (8-26 g) force categories of the von Frey filaments for primary, secondary, and tertiary regions. Data represent the mean ± S. E. of 16-17 rats (*$p < 0.05$ for young vs aged).

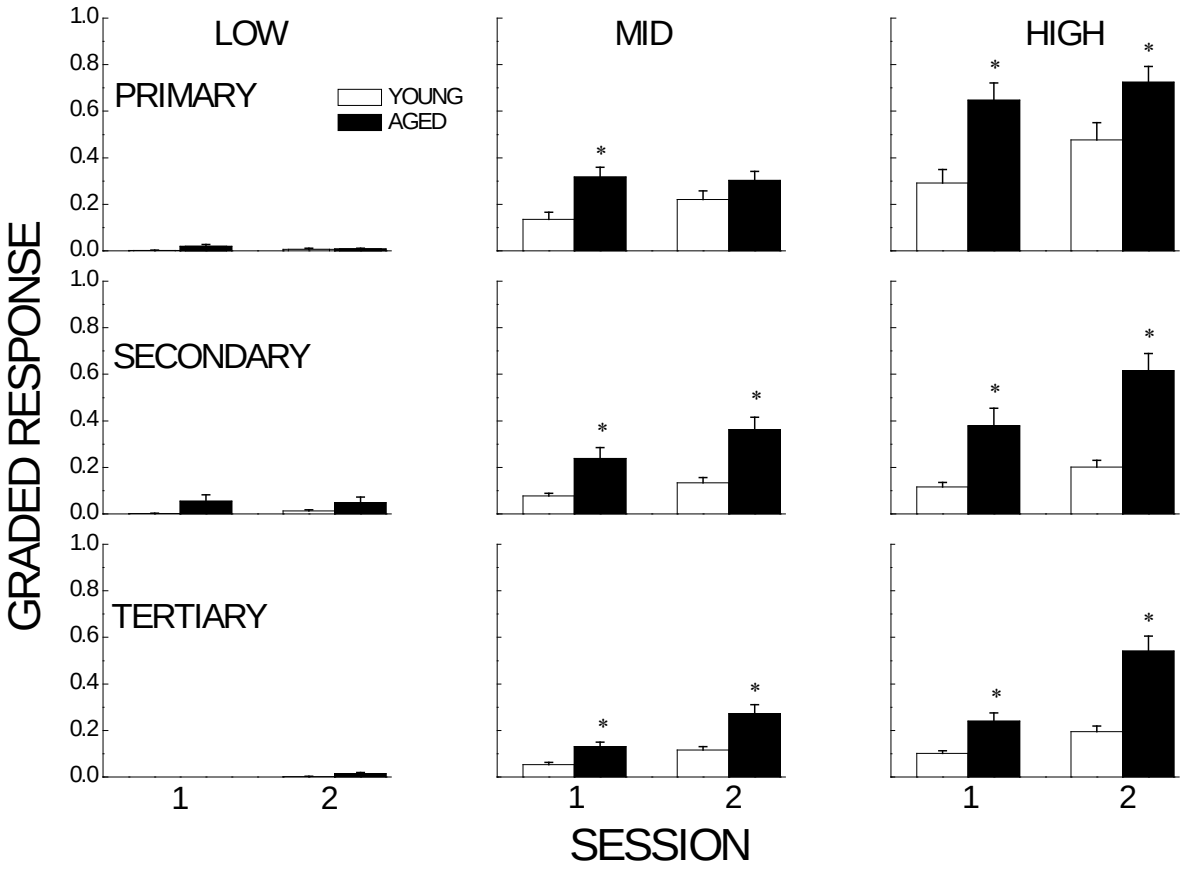

LOW
MID
HIGH
PRIMARY
SECONDARY
TERTIARY
YOUNG
AGED
GRADED RESPONSE
SESSION
1.0
0.8
0.6
0.4
0.2
0.0
1
2
*

Figure 4. The effect of age and von Frey filament stimulation on NR1 expression at the spinal cord L_1 level in Fischer 344 rats. Densitometry of NR1 expression (relative to actin expression) in young (n=17) vs aged (n=15) for control rats and young (n=17) vs aged (n=15) tested rats (top panel). Representative blots for NR1 and actin expression in the four experimental groups are also provided (bottom panel). The value for each rat is the mean of 2-4 replications on separate western blots († $p < 0.05$ for control vs tested; YC, young control; YT, young tested; AC, aged control; and AT, aged tested).

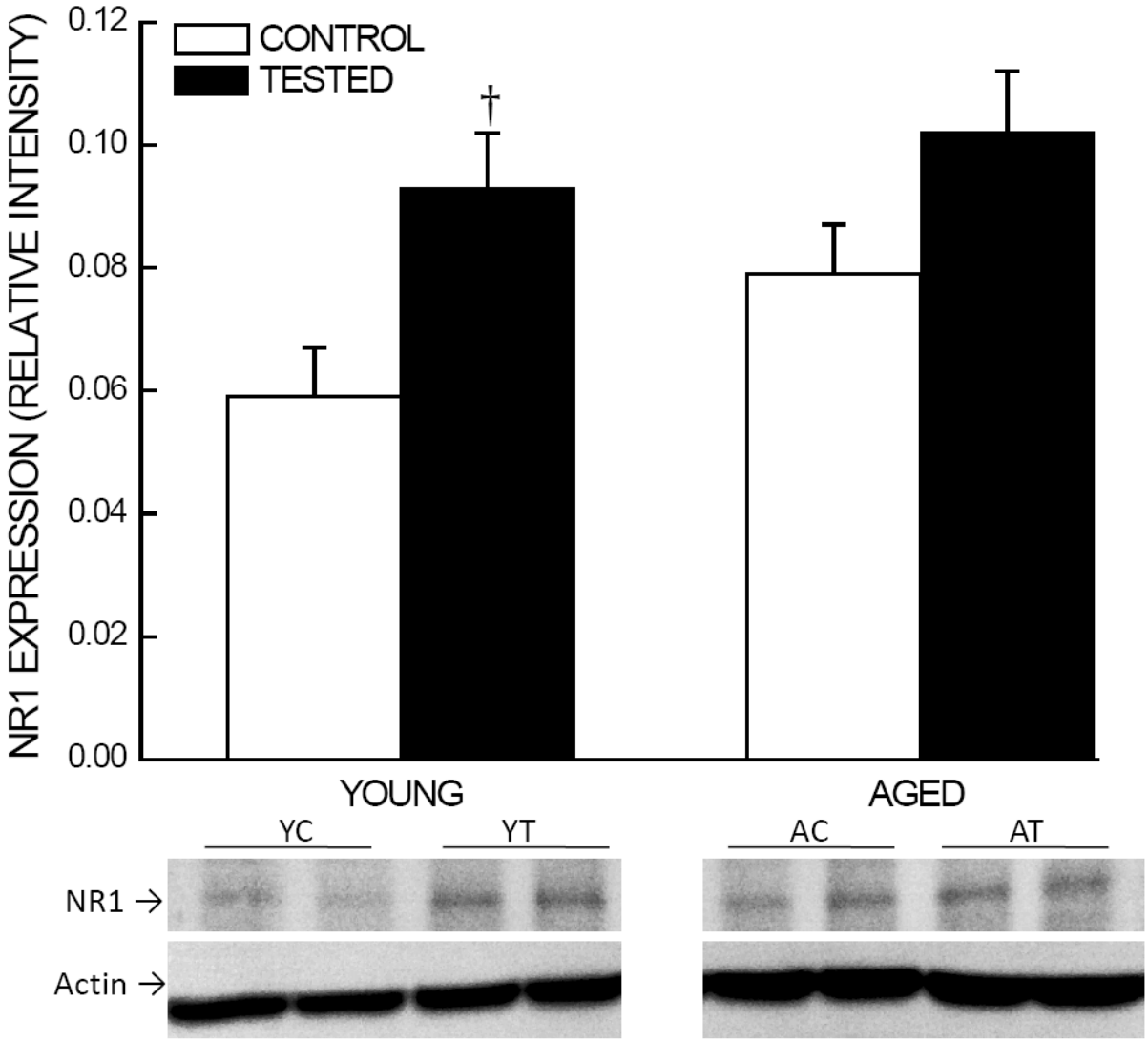

NR1 EXPRESSION (RELATIVE INTENSITY)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
CONTROL
TESTED
†
YOUNG
AGED
YC
YT
AC
AT
NR1 →
Actin →

Figure 5. NR1 expression (relative intensity) in young vs aged control (no stimulation) and tested (exposed to von Frey filament stimulation) in Fischer 344 rats. The results are expressed as individual data points overlayed with Box plots showing medians and 25-75th and 5-95th percentiles († $p < 0.05$ for control vs tested; *$p < 0.05$ for young vs aged).

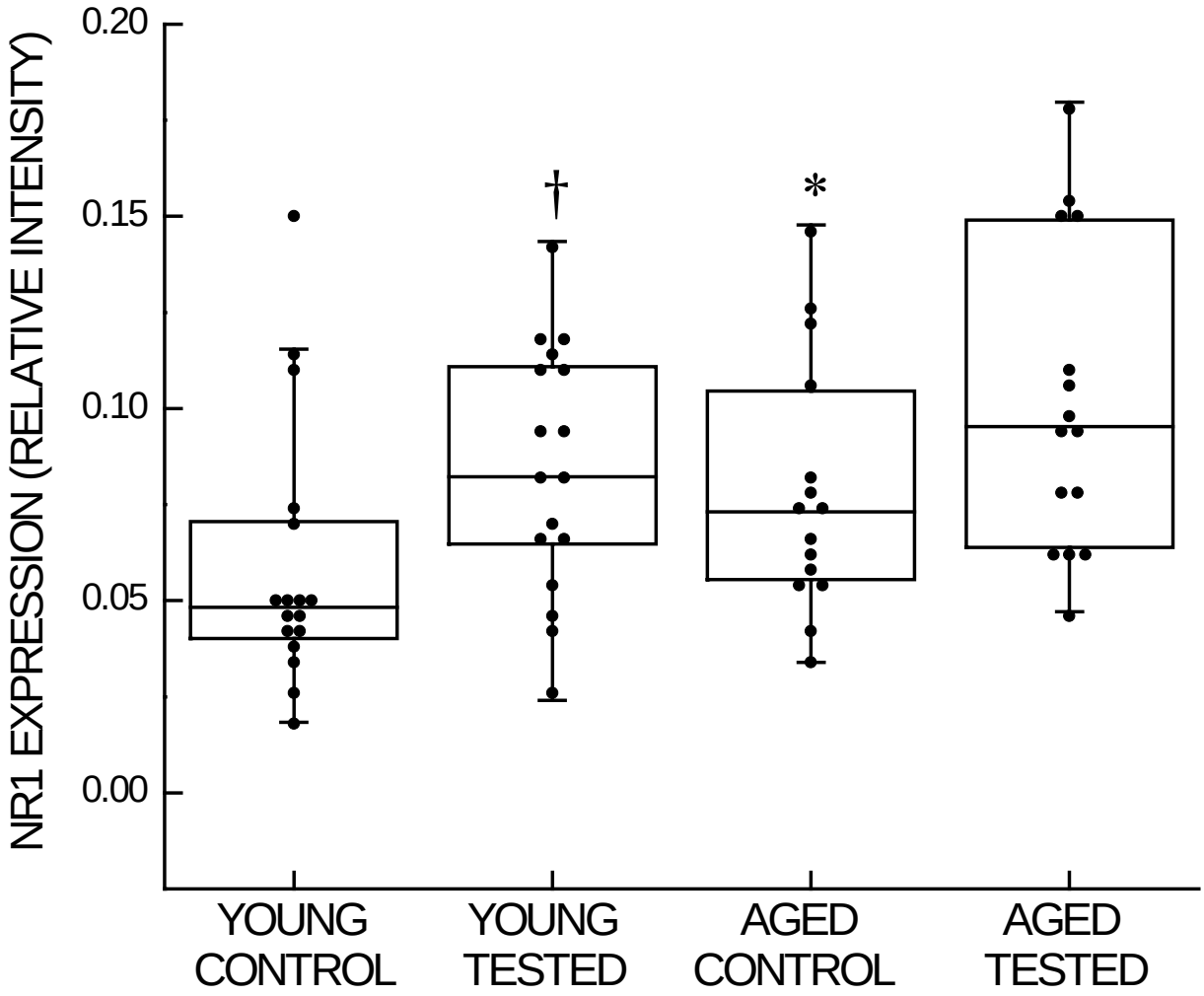
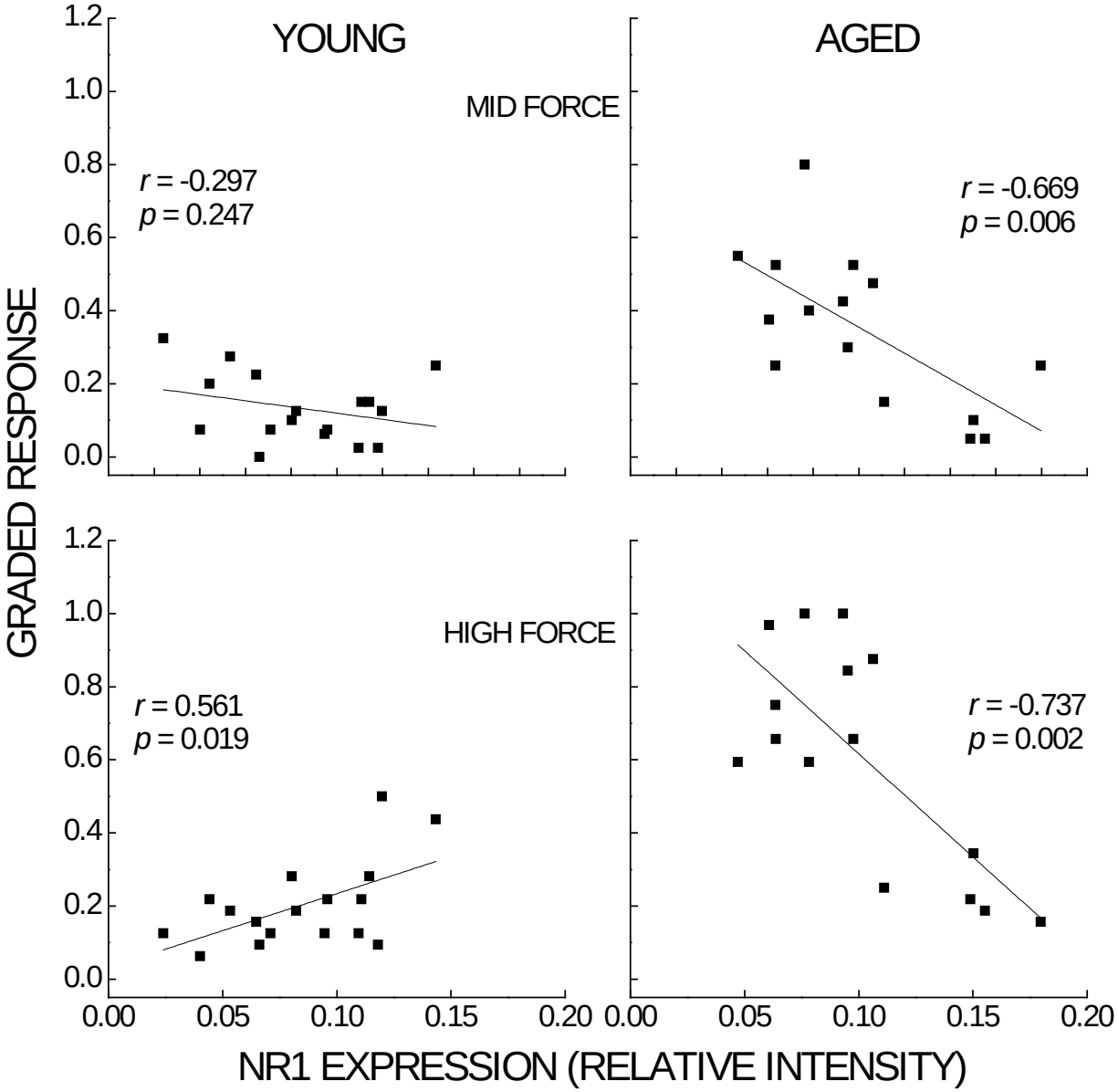

NR1 EXPRESSION (RELATIVE INTENSITY)
0.20
0.15
0.10
0.05
0.00
†
*
YOUNG CONTROL
YOUNG TESTED
AGED CONTROL
AGED TESTED

Table 1. Pearson correlation coefficients denoting association between NR1 immunostaining and graded response of three regions (primary, secondary, and tertiary) for session 1 and 2 in three filament groupings (low, mid, and high). Tertiary region, session 1, low force correlations not determined (N.D.).

			Young		Aged	
Region	Session	Force	Pearson *r*	*p*	Pearson *r*	*p*
Primary	1	Low	N.D	N.D	-0.361	0.186
		Mid	-0.237	0.361	-0.477	0.072
		High	-0.362	0.154	-0.639	0.010
	2	Low	N.D.	N.D.	-0.165	0.557
		Mid	-0.332	0.194	-0.654	0.010
		High	-0.152	0.562	-0.726	0.002
Secondary	1	Low	N.D	N.D	-0.322	0.242
		Mid	-0.042	0.872	-0.554	0.032
		High	0.277	0.281	-0.415	0.124
	2	Low	-0.220	0.396	-0.086	0.760
		Mid	-0.297	0.247	-0.669	0.006
		High	0.561	0.019	-0.737	0.002
Tertiary	1	Low	N.D	N.D.	N.D.	N.D.
		Mid	-0.227	0.380	-0.618	0.014
		High	-0.198	0.447	-0.544	0.036
	2	Low	-0.312	0.223	-0.297	0.283
		Mid	0.405	0.107	-0.577	0.025
		High	0.148	0.571	-0.492	0.062

Figure 6. Scatter plots depicting association between session 2 graded response and NR1 expression in Fisher 344 rats. Left panels illustrate lack of association between graded response and NR1 expression in young rats (n=17) for mid force (top left) or high force von Frey filaments (bottom left). Right panels illustrate strong association between graded response and NR1 expression in aged rats (n=15) for mid force (top right) or high force von Frey filaments (bottom right).


YOUNG
AGED
MID FORCE
HIGH FORCE
r = -0.297
p = 0.247
r = -0.669
p = 0.006
r = 0.561
p = 0.019
r = -0.737
p = 0.002
GRADED RESPONSE
NR1 EXPRESSION (RELATIVE INTENSITY)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0.00
0.05
0.10
0.15
0.20

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CHAPTER V

DISCUSSION

Aging and postoperative pain

Aging is associated with physiological changes that impact perioperative care and, in particular, postoperative pain and recovery. Frailty due to co-morbidities associated with aging (Taylor et al. 2005) and decline in organ function (Buchner and Wagner 1992) leave the elderly at a higher risk for postoperative complications. Age-related reduction in capacity of the neuroendocrine, respiratory, cardiovascular, gastrointestinal, and central nervous system limits the effective response to surgical pain and negatively impacts the recovery process (Aubrun 2005; Richardson and Bresland 1998).

Accurate assessment of postoperative pain in the elderly can be challenging. The commonly used visual analogue scale (VAS) may be difficult for elderly to use, resulting in improper assessment of the actual pain (Gagliese et al. 2005). In studies that compared young to old for similar surgical procedures, both groups tend to have similar VAS scores but the elderly used less analgesics (Woodhouse and Mather 1997) and self-reported less intense pain (Gagliese et al. 2005). Elderly are less likely to label a noxious stimulus as unpleasant, have lower anxiety scores and exhibit greater stoicism (Chakour et al. 1996; Pickering et al. 2002; Washington, Gibson, and Helme 2000). Thus, the intensity of pain in older individuals is similar to young but the elderly use less strong labels to describe the discomfort and exhibit less anxiety.

Despite the similar intensity of physical pain in the young and old, lower requirements for analgesia in the elderly can be due to altered age-related changes in pharmacokinetics and pharmacodynamics, e.g. extended duration of action for opioids (Aubrun et al. 2002; Moore et al. 1990), decreased volume of distribution (Owen et al. 1983), change in opioid receptor number and/or binding affinity (Ueno et al. 1988).

Inadequate treatment of postoperative pain in the elderly remains a significant clinical issue (Lynch et al. 1997; Maxam-Moore, Wilkie, and Woods 1994; Morrison and Siu 2000; Taylor et al. 2005). Basic science has developed experimental models of postoperative pain to study mechanisms and treatment modalities (cf. Table 1, Chapter II) but none have been adapted to research age-related changes in postoperative pain. In Chapter III, we addressed the need for a clinically relevant model to study effects of age on postoperative pain.

The impact of aging on primary and secondary hyperalgesia

Activation of peripheral nociceptors induces release of substance P and glutamate in the spinal cord dorsal horn (Cao et al. 1998; Li and Zhuo 2001) activating N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and kainate receptors on postsynaptic neurons (De Biasi and Rustioni 1988; Dougherty et al. 1992). NMDA receptor activation is integral to initiation and sustainment of central sensitization when nociceptive activity is enhanced (Liu and Sandkuhler 1998). Age-associated decreases in neurotransmitters, e.g. serotonin, norepinephrine, glutamate, glycine (Amenta, Zaccheo, and Collier 1991; Ko et al. 1997; Spokes 1979; Virgili et al. 2001), within the nociceptive pathways may result in altered pain perception (Iwata et al. 2002).

Injury induced by surgical incision and tissue manipulation results in primary sensitization of peripheral nociceptors (Pogatzki, Niemeier, and Brennan 2002; Zahn and Brennan 1999a). Primary sensitization refers to sensitization of peripheral afferent fibers and is characterized by a lower response threshold, an increased response magnitude to suprathreshold stimuli, increased spontaneous activity and increased receptive field size (Campbell et al. 1988; LaMotte et al. 1982; Thalhammer and LaMotte 1982). These changes result in primary hyperalgesia. Age-related changes within the peripheral nervous system, e.g. decreases in number and density of myelinated and unmyelinated fibers (Ceballos et al. 1999), lower conduction velocities (Chase et al. 1992; Dorfman and Bosley 1979), and decreased axonal transport of trophic factors (Stromska and Ochs 1982) may have contributed to lack of primary hyperalgesia in the aged rats (cf. Chapter III). Elderly patients report equivalent pain scores as young adults for the same procedure indicating similar physical intensity of pain (Woodhouse and Mather 1997). Most surgical procedures on deeper layers of tissue, muscle, and internal organs involve complex nociceptive pathways that contribute to the milieu of postoperative pain (Buvanendran et al. 2008; Buvanendran et al. 2004; Martin et al. 2004; Martin, Kahn, and Eisenach 2005). In the dorsal hairy skin model, a limited cutaneous wound is applied and thus offers a simple experimental model to commence the study of age effects on postoperative pain.

Increased afferent impulse frequency and magnitude evoked by surgical incision and tissue manipulation induce neuronal plasticity in the dorsal horn neurons (Zahn and Brennan 1999b) and modifies nociceptive pathways by enhancing and prolonging the responses to subsequent peripheral stimuli. The constant barrage of inputs from the periphery induces central sensitization in the spinal cord dorsal horn neurons manifested as secondary hyperalgesia (Pogatzki, Niemeier, and Brennan 2002; Zahn and Brennan 1999a). Age-related changes in the

central nervous system impact pain perception and processing. The wide dynamic range neurons in aged rats have significantly greater responses to heat stimuli, greater receptive field size, greater background activity and more frequent afterdischarges compared to young adult rats (Kitagawa et al. 2005). The application of an inflammatory agent prior to testing significantly increased neuronal activity in young rats. The inflammatory agent did not produce significantly greater increases in activity in the aged group (Kitagawa et al. 2005) resulting the appearance of a chronic, hyperexcitable inflammatory state. Greater neuronal activity present in the aged rat impedes the ability to encode for increased afferent nociceptive activity.

The aged rats in our studies (Chapter III) had greater baseline graded responses to nociceptive stimuli than the young rats. This inherent baseline difference may be attributed to greater neuronal response to stimuli, greater receptive field size, and greater background activity (Kitagawa et al. 2005). In addition, the injury caused by a cutaneous surgical incision may not be severe enough and therefore incapable of further increasing afferent nociceptive activity to induce central sensitization, and develop secondary hyperalgesia in the aged rat. This may contribute to the lack of secondary hyperalgesia seen in the aged rats in our initial studies.

However, the young rats did not develop secondary hyperalgesia in response to surgical incision. The young rats in this study were 5-7 months old compared to the 1.5 month old Sprague-Dawley rats in the Duarte model (Duarte et al. 2005). If age is indeed involved in lack of secondary hyperalgesia, the onset may be relatively early in the lifespan of the rat. Further studies are needed to determine if lack of secondary hyperalgesia is due to differences in age and/or strain.

In Chapter II, the currently available rodent postoperative pain models were reviewed. These pain models were developed mainly for male Sprague-Dawley rats (Brennan,

Vandermeulen, and Gebhart 1996; Buvanendran et al. 2008; Buvanendran et al. 2004; Duarte et al. 2005; Flatters 2008; Houghton, Hewitt, and Westlund 1997; Massie et al. 2004; Pogatzki, Niemeier, and Brennan 2002). The other studies used models with female Sprague-Dawley rats (Tong, Conklin, and Eisenach 2006; Weber et al. 2005), male Wistar rats (Liles and Flecknell 1993; Nara et al. 2001), female Wistar rats (Lascelles et al. 1995), male Fischer 344 rats (Martin et al. 2004), and C57BL/6 mice (Minville et al. 2008). In models with Fischer 344 rats, vertical activity, ambulation, stereotypy, conditioned operant responding to evaluate effects of laparotomy with intestinal manipulation on pain, cognitive impairment, and sedation (drug effects) were measured (Martin et al. 2004; Martin, Kahn, and Eisenach 2005). No reasonable comparisons can be made between this model and the model presented in Chapter II due to differences in surgical procedure and postoperative testing modality. Comparison of the remaining models and rat strains is difficult; any conclusions must respect the differences in surgical procedures performed and the multitude of postoperative pain testing modalities. The dorsal hairy skin incision is a viable model to study age-related effects on postoperative pain for its ease of surgery, reproducibility of results, and the mild form of nociceptive testing. However, future studies are required to determine an optimal rat strain for the experimental pain model.

Aging and descending modulatory pain pathways

A major finding presented in Chapter III was the disparity in baseline values of graded responses between young and aged rats. This phenomenon was further examined in the studies described in Chapter IV with focus on spinal cord NMDA receptor involvement. Figure 1 depicts age-related changes in pain processing.

Afferent nociceptive inputs activate descending modulatory pathways which facilitate nociception after injury but convert to predominantly inhibitory modulation with continuous noxious input (Herrero and Cervero 1996; Ren and Dubner 1996). These endogenous pathways are very effective in the young adult to reduce pain experienced upon subsequent exposure to a painful stimulus (Washington, Gibson, and Helme 2000). Descending inhibitory and excitatory neuronal controls are depicted in Figure 2. Descending modulatory pathways are subject to degeneration with age. In the aged adult, the less effective descending pathways allow for greater afferent traffic into the CNS (Edwards, Fillingim, and Ness 2003; Pickering et al. 2002; Washington, Gibson, and Helme 2000). This is also reported in aged rats where blockade of descending inhibitory pathways resulted in little increase in afferent activity versus a dramatic increase in afferent activity of young rats following thermal noxious stimuli (Iwata et al. 2002).

The endogenous modulatory pain pathways are comprised primarily of serotonergic and adrenergic nerve fibers. In aged rats, fewer numbers and aberrant morphology of serotonergic and adrenergic fibers in the spinal cord dorsal horn were reported (Iwata et al. 2002). In the same study, young rats significantly increased neuronal responses to heat stimuli after blockade of descending inhibitory pathways. This increase was not detected in aged rats leading to the conclusion that descending inhibitory pathways were less effective at the dorsal horn (Iwata et al. 2002). Decreased efficacy of descending inhibition has been reported in human studies as well (Washington, Gibson, and Helme 2000).

Age-related degeneration in the descending modulatory pain pathways may account for disparities in graded response observed between young and aged rats. Young rats had lower baseline responses than aged rats to nociceptive testing. Both young and aged rats showed increased graded response upon subsequent testing with von Frey filaments. However, the aged

rats had greater sensitivity demonstrated by higher baseline graded responses and greater maximal graded responses to nociceptive stimuli. These results may reflect age-related degeneration of modulatory pain pathways where afferent nociceptive impulses enter the central nervous system unabated. Whereas in young rats, intact descending modulatory pain pathways function to limit or 'dampen' these afferent inputs. This dampening effect is seen as the lower initial graded response and a lower maximal graded response in session 1 and session 2.

We hypothesized that spinal cord NR1 subunit expression would increase in response to nociceptive stimulation and age-related differences in magnitude of NR1 expression would be evident. NMDA receptor NR1 subunit expression has been reported to increase in response to neuropathic injury (Gu et al. 2008; Roh et al. 2008) but not increase with inflammatory (Caudle et al. 2005; Zou, Lin, and Willis 2002) or noxious heat pain (Brenner et al. 2004). A recent study reported an increase in NR1 expression in the spinal cord correlated with increased paw withdrawal frequency to von Frey filament stimulation (Roh et al. 2008). Indeed, in our study, young rats subjected to nociceptive stimulation had a significant increase in NR1 expression. The increase in NR1 expression positively correlated with an increase in graded response. However, this positive correlation was present in only one of 18 region/session/force categories analyzed. The von Frey filaments used in this study (0.07-26 g) are considered mild nociceptive stimuli. A stronger nociceptive stimulus may have yielded more positive correlations in a greater number of region/session/force categories.

An interesting finding from this study on aged rats was a decrease in spinal cord NR1 expression that negatively correlated with the increased graded response. This strong negative correlation (r = -0.554 to -0.737) was present in 9 of 18 session/region/force categories analyzed. The low NR1 expression correlated with an increased graded response lends support to the

hypothesis that the NMDA receptor is involved in age-related enhanced graded response to nociceptive testing.

Low levels of glutamate at the rostral ventral medulla (RVM), low stimulation intensities (Zhuo and Gebhart 1992, 1997) early in the injury process (Terayama, Dubner, and Ren 2002) are associated with facilitation of the descending pathways. Higher levels of glutamate, high stimulation intensities (Zhuo and Gebhart 1992, 1997), and a delay after initial injury (Terayama, Dubner, and Ren 2002) are characteristics associated with predominantly inhibitory effects from the RVM descending pathways. Most studies of descending modulatory pathways are conducted on young adult rats. None to date have reported the effects of age on the facilitatory component of the descending modulatory pain pathways. Perhaps the degeneration of endogenous pathways affects the inhibitory arm which leaves the facilitatory effects to predominate. Descending facilitatory activity may contribute to the paradoxical negative correlation of decreased NR1 subunit expression and an increase in graded response observed in the aged rats. This result requires further study to unravel the reason for the disparity.

While significant age-related differences in sensitivity and maximal graded response were reported in these studies, there is lack of evidence to support age-related divergence in sensitization. Both young and aged rats increased maximal graded response from session 1 to session 2. Despite the greater initial graded response and maximal graded response in the aged rats, the data do not support age-related differences. In this study, the testing was performed during three sessions; the data from the first two sessions were used in the analysis. In the postincisional pain study (Chapter III), we conducted 5 baseline sessions over several days. In the ipsilateral secondary area, the aged rats appeared to have greater sensitization than the young

rats. However, this was not confirmed by the statistical analysis. Resolution of this issue would require further study.

Summary

More effective therapy is needed to alleviate postoperative pain in the elderly and reduce the negative consequences of unrelieved pain. A model to research the effect of age on postoperative pain is presented. Young rats manifest primary hyperalgesia lasting through postoperative day 8 whereas aged rats did not develop primary hyperalgesia. Aged rats are more sensitive to mechanical nociceptive stimuli than young rats. Both young and aged rats exhibit sensitization. Age-related differences in NR1 expression in response to nociceptive stimulation are apparent and differentially correlate with graded response. As with many original research projects this is a work in progress. Further studies are needed to evaluate lack of secondary hyperalgesia, particularly in the young rats. The effect of age-related changes in the peripheral nervous system and how this impacts postoperative pain in the elderly is another avenue to study. Finally, and perhaps most daunting is to elucidate age-related effects of the descending modulatory pain pathways in postoperative pain. Research focused on these areas could lead to more effective methods for treatment of postoperative pain in the elderly.

Figure 1. Impact of aging on primary and secondary hyperalgesia. Aδ and C afferent fibers transmit nociceptive activity to spinal cord dorsal horn. Aβ afferent fibers transmit non-noxious sensory information to spinal cord dorsal horn neurons. Aδ and C fibers synapse on nociceptive specific neurons in laminae I and II and on wide dynamic range neurons in lamina V. Second order neurons transmit nociceptive information to the thalamus where further processing occurs. Third order neurons carry impulses to the somatosensory cortex. Some afferent input by the second order neurons is transmitted to the RVM, PBN, and PAG. Activated PAG and PBN send inputs to the RVM which stimulates the descending modulatory pain pathways. 5HT released at the level of nociceptive input to the spinal cord dorsal horn acts to facilitate or inhibit afferent nociceptive activity. (A) Peripheral age-related changes in pain pathways include Aδ neuronal degeneration, decreased conduction velocity, and decreased axonal transport. (B) In the dorsal horn, age-related changes include decreased neurotransmitter release, decreased receptor density, increased spontaneous neuronal activity and afterdischarges. (C) Descending modulatory pain pathways affected by degenerative changes—serotonergic and adrenergic fiber degeneration, aberrant morphology and decreased fiber number in spinal cord dorsal horn. Abbreviations: PAG, periaqueductal gray; PBN, parabrachial nucleus; RVM rostral ventral medulla; WDR, wide dynamic range neuron; 5HT, serotonin.

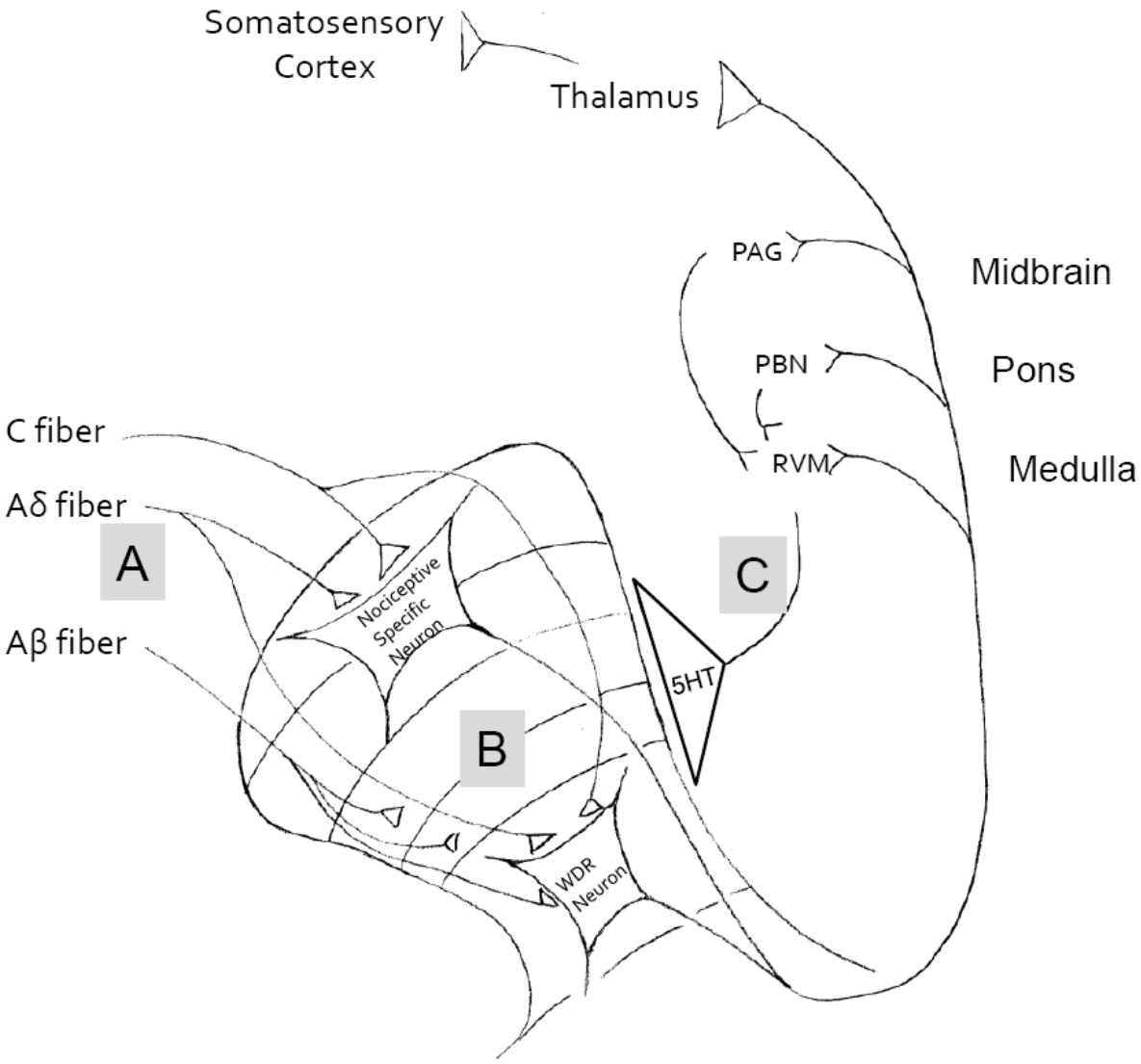
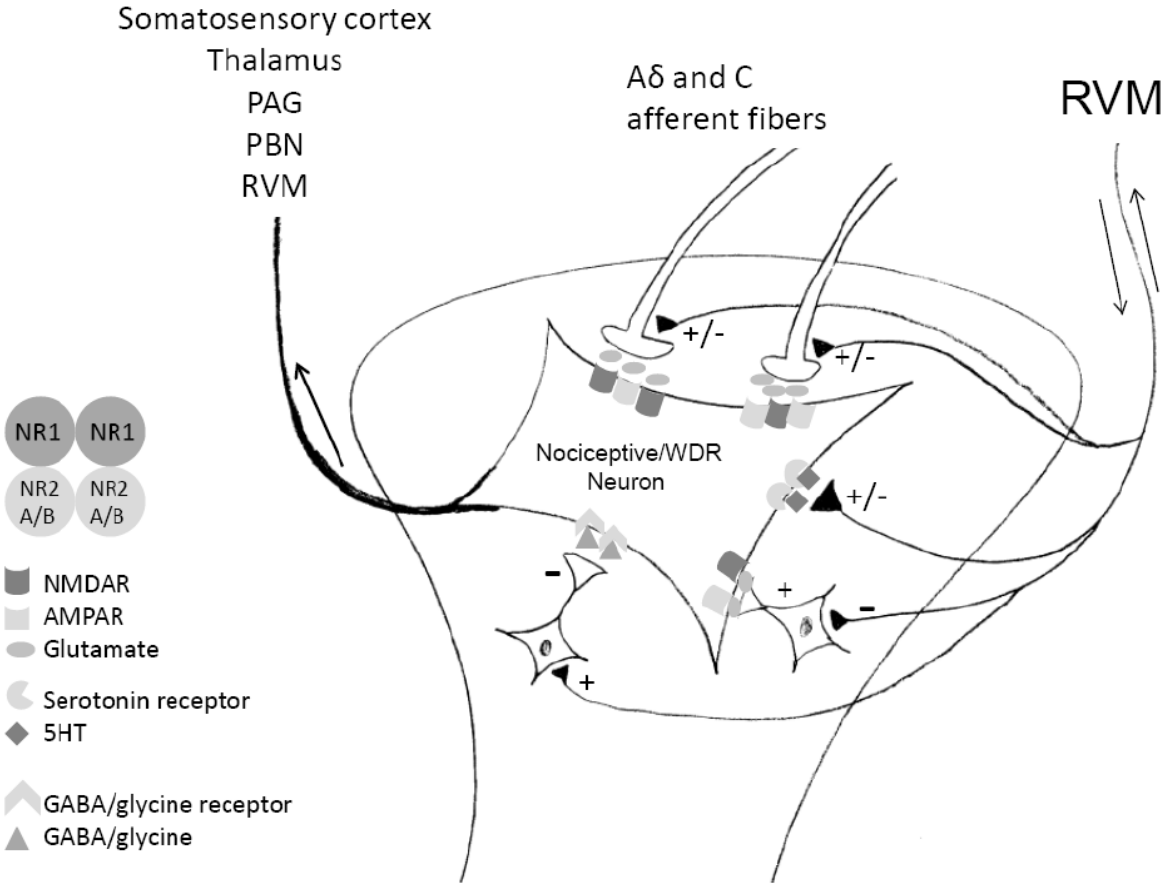

Somatosensory Cortex
Thalamus
PAG
Midbrain
PBN
Pons
RVM
Medulla
C fiber
Aδ fiber
Aβ fiber
A
B
C
Nociceptive Specific Neuron
WDR Neuron
5HT

Figure 2. Aging and descending modulatory pain pathways. Aδ and C afferent fibers synapse on second order nociceptive and/or WDR neurons in spinal cord dorsal horn. Second order neurons transmit afferent nociceptive impulses to higher brain centers RVM, PBN, PAG, thalamus, and somatosensory cortex. The RVM receives afferent activity from and sends impulses to the dorsal horn (indicated by arrows). Impulses from RVM can facilitate (+) or inhibit (-) activity of Aδ and C afferent fibers. RVM neuronal input can facilitate or inhibit activity of second order neurons, inhibit excitatory (+) interneurons, and/or excite inhibitory interneurons (-) resulting in modulation of dorsal horn nociceptive activity. Increased nociceptive stimuli increases NR1 in young rats. In aged rats, degeneration of modulatory (inhibitory effects) pain pathways results in an increase in NR1 expression. With increased nociceptive stimulation, aging results in a dysfunctional adaptation to pain processing by impeding the ability to increase NR1 expression by all aged rats. Abbreviations: PAG, periaqueductal gray; PBN, parabrachial nucleus; RVM rostral ventral medulla; WDR, wide dynamic range neuron; NMDAR, N-methyl-D-aspartate receptor; AMPAR, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; 5HT, serotonin; and GABA, gamma-amino-butyric acid.


Somatosensory cortex
Thalamus
PAG
PBN
RVM
Aδ and C
afferent fibers
RVM
+/-
+/-
Nociceptive/WDR
Neuron
+/-
-
+
+
-
NR1
NR1
NR2
A/B
NR2
A/B
NMDAR
AMPAR
Glutamate
Serotonin receptor
5HT
GABA/glycine receptor
GABA/glycine

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