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Abstract

Objective. Preclinical studies of pain and aging represent an area of research where considerations of age, strain, gender, and method of behavioral assessment are but some of the challenges that must be addressed. The results of studies related to the impact of age on pain sensitivity have ranged from increased to decreased sensitivity to no change. Examining the design of these studies one discovers that cross-sectional designs using animals of different ages have been used to evaluate age-related effects in normal animals as well as animals with inflammatory and neuropathic pain conditions. In the present review a summary of these studies is presented along with a discussion of potential mechanisms responsible for changes that have been described.

Outcome Measures. The dominant method of behavioral assessment in the majority of studies involving rodents has been reflex-based strategies that unfortunately do not reveal the same effects of experimental manipulations known to affect pain sensitivity in humans. A comparison of results obtained with reflex-based methods versus those obtained with cortically dependent operant methods reveals significant differences.

Conclusions. Increases in pain sensitivity under different experimental conditions have been suggested to result from age-related anatomical, physiological, and biochemical changes as well as compensatory changes in homeostatic mechanisms and intrinsic plasticity of somatosensory pathways involved in the processing and perception of pain. Other factors that may contribute to the impact of age on pain sensitivity include dysregulation of the hypothalamic-pituitary-adrenal axis and changes in autonomic function that occur with advancing age. In the future translational research in the field of pain and aging will need to focus on establishing clinically relevant animal models and assessment strategies to evaluate the causal relationships between the biological changes associated with advancing age and the varied behavioral changes in pain sensitivity.

Key Words. Inflammation; Neuropathic; Reflex Behavior; Operant Behavior; Microglia; Central Sensitization; Plasticity

Introduction

Age-Related Changes in the Somatosensory System

How does advancing age impact biological systems responsible for the experience of pain? The answer to this question is not as simple as one might expect as chronic pain in the elderly is a far more complex condition clinically, biologically, psychologically, and therapeutically than pain in younger segments of the population [1–4]. That being said, it is well documented that sensitivity in sensory systems decreases with advancing age for hearing, taste, smell, vision, and touch due in part to diminished numbers of specialized peripheral receptors combined with a deterioration of supporting tissues [5].

Consistent with other sensory modalities, numerous age-related anatomical and functional changes have been documented in both human and animal studies for the somatosensory system [1]. For example, peripheral nerves show a reduction of myelinated and unmyelinated fibers [6–8] as well as signs of damage, including Wallerian degeneration [7,9]. The number and size of sensory neurons in dorsal root ganglia also increases throughout early adulthood, peaks at midlife (13–18 months), and then decreases thereafter [10,11]. Age-related reduction in the number of peripheral afferents, the presence of demyelination together with increasing inflammation are strikingly similar to the pathological changes that occur following nerve injury in younger animals [1,11–17]. It is therefore suggested that there may be mechanistic similarities between the pathophysiological changes underlying the emergence of neuropathic pain and those...
associated with age-related changes in nociception. Another interesting parallel is the cellular and molecular mechanisms responsible for the development of chronic pain which have an undeniable overlap with mechanisms associated with aging [18,19], thus providing the rationale for studies evaluating synergistic interactions between the biological process of aging and the pathological condition of chronic pain.

In addition to the peripheral changes in the anatomy of substrates responsible for somatosensation, altered expression of neurotransmitters and receptors is observed in the spinal cords of old animals and human postmortem material. For example, immunohistochemical studies reveal decreased labeling of calcitonin gene-related peptide, substance P, nitric oxide, and somatostatin in the dorsal horn of aged rats [20–25]. Evidence of a progressive age-related loss of serotonergic and noradrenergic terminals in the dorsal horn also suggests the potential for changes in descending modulatory pathways [13,15,25,26]. In fact, it has been suggested that age-related increases in sensitivity may be the result of plasticity in spinal nociceptive processing related to a functional impairment of descending modulatory pathways [27]. Decreased numbers of opiate receptors [28,29] and the decreased efficacy of opiate-mediated nociception [30,31] are also likely to contribute to age-related changes in the processing and perception of nociceptive information. Not to be ignored are changes in the expression and functional state of spinal glial cells (i.e., astrocytes and microglia) that add to an evolving construct of pathophysiological changes responsible for age-related changes in pain sensitivity [32–34]. Although the many anatomical changes in substrates of nociception are suggested to play a significant role in the expression of age-related changes in sensitivity, it is important not to ignore the molecular alterations in gene expression for trophic factors, neuropeptides, cell adhesion molecules, ion channels, or genes related to mitochondrial function and calcium handling as additional contributing factors [35]. Establishing causal relationships between the varied changes mentioned above and the functional components responsible for pain sensations represents one of the many challenges in pain and aging research. Overcoming these challenges will have a significant impact on the development of novel therapeutic interventions.

In spite of efforts to establish a relationship between pain and aging, this area of research has not attracted widespread interest. In fact, one is hard pressed to find systematic, long-term longitudinal evaluations of age effects (in animals or humans) related to either the behavioral consequences or underlying peripheral and/or central mechanisms associated with advancing age and the experience of pain. The main findings from studies on pain sensitivity in humans include an increased threshold and decreased tolerance with advancing age [1,14,36,37]. These results have been interpreted in terms of age exerting opposite influences on the sensory dimension of pain (pain detection threshold) vs the affective dimension (tolerance threshold) [38–40]. Alternatively, reduced peripheral input with age may reduce sensitivity to non-nociceptive and mildly nociceptive stimulation (at or below pain detection threshold), whereas increased excitability within intact pain pathways could increase the magnitude of suprathreshold pain sensations.

Understanding the biological process of aging and its impact on the perception of nociceptive information requires the development of animal models that simulate pathophysiological conditions associated with advancing age. Elucidation of relationships between biological changes and pain sensitivity that occur during aging also requires behavioral methods to monitor changing sensibilities across the life span of experimental animals. These methods must translate to human studies of pain processing. For this reason, the translational validity of behavioral assessment methods is critical to establishing clinically relevant models that will provide a vehicle for determining the mechanisms responsible for age-related changes in sensory processing, identifying potential therapeutic targets, and testing the efficacy of novel interventions.

Challenges Related to Studying the Preclinical Effects of Age on Pain Sensitivity

Animal studies examining the effects of age on pain sensitivity have resulted in conflicting observations that include increases, decreases, or no change in cutaneous sensibilities with advancing age [1]. It is important to point out that the majority of these studies employed reflex-based behavioral measures to determine changes in thermal and/or mechanical sensitivity. Unfortunately, these methods do not reveal the same effects of experimental manipulations known to affect pain sensitivity in humans [41–49]. That being said, the majority of published evidence from clinical studies supports the conclusion that neurochemical and neuroanatomical changes taking place in midlife alters the response to tonic and chronic painful stimuli later in life. Importantly, the prevalence of many common pain problems in humans tend to peak in midlife and the majority of patients referred to pain clinics are between 40 and 60 years of age. Helping explain these findings are reports that advancing age results in degeneration of endogenous inhibitory systems and increasing cell death that may increase susceptibility to neuropathies and myofascial pain disorders [10,11,50]. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is also associated with changes in autonomic function, dysfunctional psychosocial interactions, genetic and gender differences, and the increased prevalence of autoimmune disorders that have painful components such as rheumatoid and osteoarthritis, autoimmune polyneuropathies, and multiple sclerosis. All of the various systems contributing to these conditions are part of a complex supersystem that has a significant influence on the processing of nociceptive information and the ultimate perception of pain [51]. Unfortunately, little direct evidence exists, supporting the causal nature of specific relationships with chronic pain, thus revealing many important questions that have long been ignored.
Another challenge to studying age-dependent changes in pain processing is the selection of a clinical condition to serve as the model for preclinical studies. A number of pain conditions emerge from those encountered with advancing age, including those associated with: 1) arthritis; 2) nerve injury; 3) visceral structures; 4) postoperative procedures; 5) stroke; and 6) musculoskeletal conditions. In many instances, more than one of these conditions exists, making it even more challenging to create a preclinical model reflecting the complexity of the human condition.

Behavioral Assessment of Age-Related Changes in Pain Sensitivity

An important decision in the study of pain in “old” rats is the selection of an assessment strategy to evaluate responses to nociceptive and non-nociceptive stimuli. There are many options to choose from, including innate reflex responses, complex learned behavioral tasks, spontaneous behaviors, daily tasks like sleeping and grooming, and the interruption of ongoing behaviors. Previous studies in rodents have attempted to relate age-dependent changes in reflexive behaviors (e.g., tail flick, hindpaw withdrawal, licking/guarding) to human pain sensitivity. However, all reflex responses are mediated by spinal and/or spinal-brainstem/spinal circuits, in contrast to complex behavioral tasks that rely on processing of sensory information throughout the neuraxis, including the cerebral cortex [44,45,49]. Cortically dependent strategies of behavioral assessment rely on neural structures important to the general perception of pain and measure the impact of peripheral stimuli on clinically relevant measures of cutaneous sensibilities [43–45,49]. Due to the fact that pain is a cortically dependent sensation, it should be easy to understand that if one is to study pain, it is essential to activate the neural substrate(s) responsible for the sensation being studied. Due to the varied results of different studies focusing on pain and aging, it has been recommended that the relationship between nociception and age should be studied using multiple stimulation modalities and pain outcome measures, including cortically dependent integrated behaviors [52].

Effects of Age on Pain Sensitivity: Preclinical Studies

A review of studies using reflex-based assessment strategies to examine age-related changes in pain sensitivity reveals a confusing inconsistency of results. Hess et al. [29], using latency of responses to paw lick and tail flick in young (2–3 months), adult (6–12 months), and aged (24 months) rats, described a decrease in thermal and electric shock sensitivity with increasing age that correlated with a decrease in the number of opiate receptors in the frontal poles, striatum, and hippocampus. Wang et al. [53], using thermal response latencies in mice, showed that young animals (6–8 weeks) had significantly shorter response latencies than animals 24 months of age. This decreased sensitivity in older animals was greater for females than males and correlated with a decrease in the expression of Nav1.8 sodium channels, thus providing a mechanistic explanation for the decrease in thermal sensitivity. Similar results in mice were described by Wang and Albers [35] who showed using response latencies to thermal stimuli that aged male and female mice had decreased sensitivity to thermal stimuli. These results were complemented by observations of a decrease in receptor expression for the growth factor artemin and the ion channel TRPV1. Contrary to these results are those of Chan and Lai [54] who showed a decrease in response latencies (i.e., increased sensitivity) using hot plate latencies for animals 1.6 to 11.2 months old. This study also showed an age-dependent decrease in the therapeutic efficacy of morphine and clonidine. Pickering et al. [55] described an age-dependent increase in sensitivity to acute pain in the rat. Consistent with these results are those reported by Iwata et al. [27] who described an increase in thermal sensitivity for rats 7–13 months (adult) vs those 29–34 months (aged) when measuring licking and guarding behavior. These results correlated with the response profile of wide dynamic range and nociceptive specific neurons recorded from these two groups of animals. Neurons recorded in adult animals had significantly lower mean background activity and after-discharge responses compared with aged animals. Similar increases in excitability and size of receptive fields for neurons recorded in the dorsal column nuclei in aged vs adult animals has also been reported [56]. In summary, conclusions from 25 studies addressing age-related changes in pain sensitivity revealed by reflex-based behavioral responses showed decreased sensitivity (9/25), increased sensitivity (12/25), or no changes in sensitivity (4/25) with advancing age. There are many possible factors contributing to the widespread variability of results, including the role of different species, testing methods, and gender and age of animals [57].

Recently, a study was carried out where both operant escape and reflex testing methods were used to evaluate the impact of age on thermal sensitivity in the rat [58]. This was a cross-sectional study with animals ranging in age from 8 to 32 months and consisted of a comparison of results obtained with both reflex and cortically dependent escape behaviors used in the same rats under identical stimulus conditions. In this study, operant measures of pain assessment revealed an increase in thermal sensitivity to escape from heat stimulation across the life span of animals (Figure 1). By contrast, reflex responding did not show any age-related differences in sensitivity to 44.5°C (Figure 2). In the case of cold sensitivity, operant escape testing revealed increased sensitivity from 8 to 32 months. Similarly, decreased latencies for licking/guarding to a stimulus of 1.5°C were observed for animals ranging from 11 to 27 months of age (Figure 2). However, a significant reversal of behavioral effects was observed at 35 months. Thus, in direct comparisons with operant escape, reflex-based assessment tasks did not appropriately or consistently assess changes in thermal sensitivity across the life span of rats. The escape test used in this study was not sensitive to muscle weakness or other motoric influences of aging because the primary measure of sensitivity was the relative occupancy of an escape compartment, regardless of the speed of movement between compartments. However, reflexive licking and guarding
during 1.5°C stimulation may have been attenuated by physical impairments as grip strength and endurance on an inclined plane were substantially impaired for these animals at 32 months of age. These deficits and associated physical impairments at 35 months appeared to have reversed a trend toward decreased response latencies to 1.5°C at 27 months.

**Inflammatory Pain**

A potential mechanism for the increase in pain sensitivity observed with advancing age is age-related increases in systemic inflammation [59–61]. Chronic inflammation sensitizes peripheral nociceptors, is a factor in central sensitization [62], and activates central stress circuits.

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**Figure 1** Age-related responses of animals to different temperatures. Animals ranging in age from 8 to 32 months were evaluated on a thermal escape task for responses to 15°C (A), 10°C (B), 42°C (C), and 44.5°C (D). Accumulated escape durations in seconds are represented on the y-axis, and response numbers in a given session are represented on the x-axis. To control for possible effects of age on activity levels, differences between the cumulative escape durations for nociceptive stimulation and the cumulative escape durations for 36°C stimulation are plotted. For each temperature, the maximal response durations (i.e., greatest sensitivity) was observed for animals 24–32 months of age, while the lowest response durations (i.e., least sensitivity) were for the youngest animals. Published with permission from Oxford University Press.
One way to evaluate the influence of injury- or age-induced inflammation is to provide an inflammatory challenge. In a study by Zhang et al. [63], hindpaw withdrawal latencies were evaluated in adult and aged animals following injections of complete Freund's adjuvant (CFA). The results showed that aged animals had a significant increase in sensitivity over adult animals. CFA injections were also used by Kitagawa et al. [56] to show that the excitability of dorsal horn nociceptive neurons becomes sensitized with advancing age, but the excitability cannot be further increased by inflammation. In another study, Gagliese and Melzack [64] showed that formalin injections in animals 3 and 24 months of age had similar pain scores that were significantly less than animals 18 months of age, suggesting that sensitivity to tonic pain may peak at midlife. Following formalin injections, Iwata et al. [65] reported a larger number of c-fos positive cells in the medullary dorsal horn of older rats compared with their younger counterparts. This difference in neuronal activity correlated with an increase in sensitivity in the older cohort of animals.

Using an operant escape task to assess responses to thermal stimuli, Yezierski et al. [58] examined the effects of formalin injection on thermal pain sensitivity evaluated over 5 weeks of testing. Hyperalgesia was not observed for animals 8 months of age, but a significant increase in thermal sensitivity was obtained for cold and heat stimulation in 16- and 24-month-old animals (Figure 3). Hyperalgesia in response to inflammatory challenge was greater for older animals and age-related hypersensitivity for nociceptive heat and cold stimulation was only revealed by operant escape testing and not by more traditional lick/guard reflex tests. These results are consistent with those showing that paw injections of CFA in 18-month-old rats increased thermal sensitivity and expression of the peptide dynorphin (DYN) in the spinal cords of 18-month-old rats, compared with 3-month-old rats [63]. Spinal DYN has been shown to be pronociceptive, and its upregulation is required for the maintenance of neuropathic pain [66]. In a recent study, an important observation with regard to inflammatory pain showed that with repeated formalin injections over a 15-month period, there was a significant enhancement of age-related changes in sensitivity to thermal stimuli (Yezierski and Vierck, unpublished observations). The conclusion from this study was that repeated inflammatory injury results in a cumulative effect such that the long-term effects on sensitivity are greater than the normal enhancement of sensitivity due to age. This observation may be explained by age-dependent differences in the plasticity of spinal cord circuits involved in the processing of nociceptive information following injury [67].

Neuropathic Pain

Efforts to evaluate age-dependent changes in pain sensitivity following nerve injury have also been evaluated. Following sciatic nerve ligation in young (4–6 months), mature (14–16 months), and aged (24–26 months) animals, prolonged increases in thermal sensitivity were observed and these changes were present at 3 and 21 days following injury in all groups but lasted longest (35 days) for aged animals [68]. Crisp et al. [69], comparing chronic constriction injury (CCI) and partial sciatic nerve ligation (PSNL) models, showed that aged (24–26 months) rats undergoing PSNL developed a more vigorous and longer duration thermal hyperalgesia compared with their younger (4–6 months) counterparts. In another study by Chung et al. [70] using a model of L5/L6 spinal nerve ligation in young (40 days), mature (4 months), and old (15 months)
animals, it was the oldest animals that showed a decrease in sensitivity. Pickering et al. [55] also described a decrease in sensitivity to neuropathic pain for senescent (37–39 months) animals compared with old (20–22 months) and young (4–6 months) animals. To complete the variety of effects produced in preclinical models of neuropathic pain, Kim et al. [71], following partial denervation of the tail, found no differences in responses to thermal stimuli for animals 7–8 weeks vs 18 months of age, but did see significant increases in sensitivity in older animals when evaluating mechanical allodynia. In all of these studies, the behavioral assessment methods consisted of reflex-based strategies which may have contributed to the variable results. Additionally, differences in results among these studies could have been due to different ages of animals along with different experimental conditions.

Anatomical and physiological evidence for similarities between the biological process of aging and the pathological conditions associated with neuropathic pain suggests that there may be similarities in behavioral changes observed during aging and those following nerve injury. Hypersensitivity to cold stimulation is characteristic of neuropathic pain models [48], and increased sensitivity to cold was detected by lick/guard and operant escape testing of older animals. Thus, similar peripheral and spinal abnormalities could underlie the effects of CCI and aging on reflex and operant responses to cold. However, operant responsivity to heat increases with age but not after CCI, and hyporeflexia for heat is not observed with age [48]. Development of heat hyperalgesia with advancing age is therefore likely to depend upon changes within supraspinal pain pathways.

**Discussion**

In spite of the controversial results of preclinical studies, sufficient evidence does exist, supporting the conclusion that age-related increases in nociceptive sensitivity can be demonstrated under naïve, inflammatory, and neuropathic conditions. One of the potential explanations for these changes is believed to be associated with a loss of "buffering" capacity in which compensatory homeostatic mechanisms are rendered ineffective, thus leading to a permissive environment for the development of pain [72]. An important central component of this buffering mechanism is provided by microglia. The supporting, i.e., housekeeping, functions of these cells are well documented, and so to are the age-related anatomical changes in these cells that occur in various brain regions [73]. With regard to the development of chronic pain, the cellular responses of microglia to nerve/tissue injury has been suggested to be the driving force for neuronal hypersensitivity leading to the production and release of inflammatory mediators such as cytokines and chemokines [74,75]. Included in this response is the infiltration of inflammatory cells such as mast cells, neutrophils, macrophages, and T-lymphocytes that have been implicated along with microglia and astrocytes as pathological components contributing to the development of chronic pain [62].

![Cumulative Escape Duration: Responses 1-3](image)
Activated microglia have also been implicated in the initiation of chronic pain via the local release of neuroactive substances, including cytokines, adenosine triphosphate, substance P, reactive oxygen species, nitric oxide, arachidonic acid, fraktalkine, and nerve growth factors [74]. Selective inhibition of activated microglia can alleviate acute and chronic pain behaviors [75]. The microglia-to-neuron signaling link has also been shown to involve a molecular pathway in the spinal cord that includes toll-like receptors, phosphorylated mitogen-activated protein kinase, and purinergic P2X4 receptors [76,77]. Interestingly, the microglia-to-neuron signaling pathway involving PGE2 has been shown to be involved in producing excitability changes underlying chronic pain following spinal cord injury [78]. This same pathway could play a significant role in the emergence of age-related chronic pain conditions. Although astrocytic activation is less robust in older compared with younger animals, it does remain elevated for long periods of time [32,79]. Thus, substantial evidence exists in the initiation of chronic pain conditions for an immune response that includes a well-orchestrated temporal pattern of activation of different immune cells, including microglia and astrocytes.

In recent years, the “glia cascade” has also become the focus of studies related to the regulation of synaptic strength and plasticity and the generation of central sensitization [74]. The robust response of microglia to sciatic nerve injury further supports the possibility that activated microglia contribute to the functional plasticity associated with the development of chronic pain [79,80]. However, the contribution of glia to the induction or maintenance of chronic pain in aged rats is still an evolving story. Given the age-dependent changes in thermal sensitivity, an obvious question is whether there are morphological and subsequent functional changes in spinal microglia that parallel age-related changes in thermal sensitivity. Given the hypothesis that a changing phenotype at the cellular level reflects a change in function, one can assume that age-related morphological changes in microglia are likely to be part of the central mechanism responsible for the expression of age-dependent increases in pain sensitivity.

Another potential contributor to the changing sensitivity observed with advancing age are the effects of age on autonomic function. The sympathetic nervous system (SNS) is critical for maintenance of physiological homeostasis under basal conditions and the response to stress. Experimental evidence has shown that tonic whole-body SNS activity increases with age [81,82]. Although mechanisms underlying age-related increases in SNS activity are not known, a relationship between chronic pain and autonomic dysfunction is well documented, and there is a growing acceptance that changes in sympathetic tone can be instrumental in the generation and maintenance of chronic pain. A recent review [83] summarizes an extensive literature supporting the following relationships: 1) psychological stress activates limbic structures projecting to the HPA, resulting in an increase in sympathetic tone; 2) activation of stress circuitry increases pain sensitivity by central actions leading to stress-induced hyperalgesia; 3) chronic sympathetic activation and associated peripheral vasoconstriction produce muscular ischemia and a microenvironment conducive to myofascial pain; and 4) nociceptors in deep tissues are particularly sensitive to ischemia and are potent generators of central sensitization when tonically active. A key element in this sequence of events is stress. The clinical literature emphasizes the detrimental effects of psychological stressors such as anxiety or fear, but also pain and especially inflammation are significant players in the activation of limbic stress circuitry [84]. Therefore, inflammation and associated pain that increase with age are likely sources of stress, which potentiate the expression of hyperalgesia and pain. Acute tissue injury also activates a cascade of interdependent nervous, endocrine, and immune processes that collectively impact the perception of pain [51]. Understanding how these different components contribute to changes in pain sensitivity is an important area of future research.

Conclusions

There is no denying the fact that additional research is needed to more clearly define the nature of behavioral, physiological, biochemical, and molecular changes that occur with advancing age in normal and pathological conditions. Identifying specific patterns of change that occur is an essential first step that will lead to assessing underlying mechanisms. At present, a partial list of potential mechanisms responsible for age-related increases in pain sensitivity include anatomical, physiological (e.g., central sensitization), age-related plasticity, immune, neuroendocrine, inflammatory, and autonomic, with each component having its own inherent level of complexity. Without hesitation, it is easy to say that there remain numerous challenging questions for the preclinical scientist to explore. In addition to what many would refer to as mechanistic questions related to the relationship between age and pain, there remains a long list of basic and fundamental questions that include: 1) What is the impact of age-related autonomic dysfunction on pain sensitivity?; 2) What are the effects of central injury on pain sensitivity at different ages?; 3) Are there gender differences on pain sensitivity at different ages?; 4) What are the hormonal and immune influences on pain sensitivity at different ages?; and 5) What are the effects of age on the efficacy of opiates, anticonvulsants, and antidepressants? These and other questions clearly reveal that the field of pain and aging has many challenges for both clinical and preclinical studies. Commitment to defining the changes and unraveling the underlying mechanisms will hopefully result in significant advances in the management of what has become a significant clinical challenge for the fastest-growing segment of our population.

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