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Pain and Itch Processing in Aged Mice

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Abstract: Most reports agree that aging negatively impacts pain processing and that the prevalence of chronic pain increases significantly with age. To improve current therapies, it is critical that aged animals be included in preclinical studies. Here we compared sensitivities to pain and itch-provoking stimuli in naïve and injured young and aged mice. Surprisingly, we found that in the absence of injury, aged male and female mice are significantly less responsive to mechanical stimuli and, in females, also to noxious thermal (heat) stimuli. In both older male and female mice, compared to younger (6-month-old mice), we also recorded reduced pruritogen-evoked scratching. On the other hand, after nerve injury, aged mice nevertheless developed significant mechanical hypersensitivity. Interestingly, however, and in contrast to young mice, aged mice developed both ipsilateral and contralateral postinjury mechanical allodynia. In a parallel immunohistochemical analysis of microglial and astrocyte markers, we found that the ipsilateral to the contralateral ratio of nerve injury-induced expression decreased with age. That observation is consistent with our finding of contralateral hypersensitivity after nerve injury in the aged but not the young mice. We conclude that aging has opposite effects on baseline versus postinjury pain and itch processing.

***Perspective:** Aged male and female mice (22–24 months) are less sensitive to mechanical, thermal (heat), and itch-provoking stimuli than are younger mice (6 months).*

***Key Words:** Pain, itch, aging, mouse, sexual dimorphism*

Chronic pain is more prevalent in the elderly.[1-3] However, there is little consensus as to age-related differences in the mechanisms that provoke acute and chronic pain.[4,5] Preclinical studies in rodents also lack agreement. Thus, there are reports of increases, decreases, or no change in pain processing[6] in aged versus young mice. The lack of agreement is true for baseline thresholds to both thermal[7-12] and mechanical stimulations.[10,13-15]

Age-related differences in rodent nociceptive processing in the setting of tissue or nerve injury are also apparent, but the reports are inconsistent. Thus, several studies reported hyperalgesia in response to inflammatory mediators[12,14] or no change[16] in older animals. Peripheral nerve injury-induced thermal hyperalgesia[17] and mechanical allodynia[18] are also significantly greater and more prolonged in older rodents. And in 26 to 27 months old F344xBN rats, tissue injury-induced indices of inflammatory pain are significantly increased.[19] Other studies reported that geriatric animals (37–39 months) are reportedly less impacted in various neuropathic pain models compared to aged (20–22 months) or young (4–6 months) animals. Surprisingly, these mice are reportedly more sensitive in tests of acute pain.[20,21] Although the pain associated with certain diseases, notably in osteoarthritis, definitely increases with age, other studies of sensory thresholds, for example, using Quantitative sensory testing, found significant increases as a function of age.[22,23] For review, see Ref 24. And aged mice with sickle cell disease exhibit greater indices of pain compared to young mice with the same condition.[25]

Differences in genetic background, methodology (reflexive vs operant testing), and environmental factors likely explain some of the discrepancies in the literature. However, peripheral and central neuroanatomical and functional changes that occur with age undoubtedly contribute.[26] For

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example, the number of nitric oxide synthase-immunoreactive neurons in the dorsal horn of aged rodents increases significantly,[27] and levels of serotonin and norepinephrine decrease in the spinal cord of rats as they age.[28] Furthermore, baseline microglia density increases in the spinal cord of aged mice,[29] as do spinal cord levels of the proinflammatory cytokines, tumor necrosis factor alpha and interleukin 6. Given the important contribution of microglia to nerve injury-induced pain processing,[30] such changes could significantly impact pain as the animals age.

There is also evidence that traditional therapeutic approaches to treat pain are less effective in the elderly,[31] in part due to comorbid conditions. For example, impaired renal function in the elderly can exacerbate side effects associated with opiates.[32] Furthermore, altered pain modulatory circuits in the elderly could contribute to the reduced utility of opioids and other analgesics.[33] Preclinical studies have, in fact, reported that spinal opioid-induced antinociception is reduced in older rodents,[10,34] possibly due to decreased expression[9] and/or affinity[35] of opioid receptors.

Given the substantial discrepancies in the literature, here we evaluated both naïve and nerve-injured aged and young mice in a battery of behavioral tests of pain processing. We also examined the expression of several injury markers in sensory and spinal cord neurons. Importantly, although our primary focus is on aged versus young mice, as sex and gender contribute to differences in pain perception,[36] we studied both male and female mice. Finally, because pruritus is also more prevalent and difficult to treat in the elderly,[37,38] we also examined the response to pruritogens in these mice.

Methods

Animals

All animal experiments were approved by the University of California San Francisco Institutional Animal Care and Use Committee and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Based on the Jax laboratory definition of young (3–6 months), middle age (10–14 months), and aged (18–24 months), we studied separate groups of 6-month-old (young group) and 22 to 24 months (aged group) male and female C57BL/6 mice. The young and aged cohorts correspond to 20 to 30 and 56 to 69-year-old humans, respectively. The aged mice cohorts were purchased at 2 months of age from Jackson and aged in-house at University of California San Francisco. As some aged mice died during the extended duration of the aged mice experiments, there is variability in the number of mice across tests.

Behavioral Analyses and Statistics

For all behavioral tests, mice were first habituated for 1 hour in Plexiglas cylinders. All mechanical, thermal, motor, and pruritogen tests were performed as described previously.[39] Briefly, for the Hargreaves test of heat pain sensitivity, we measured latency to withdraw the hindpaw from a heat source that was applied through a glass surface. In the tail flick test of heat pain, we measured the latency of the mouse to withdraw its tail from a water bath at different temperatures (50 and 52 °C). For the hot plate test (48, 52.5, and 55 °C), we recorded the latency to lick or flinch the hindpaws or to jump. To measure mechanical paw thresholds, we placed mice into clear plastic chambers on a wire mesh grid and stimulated the hindpaw with graded von Frey filaments. We used the up-down method[40] to measure mechanical withdrawal thresholds. For cold responsiveness, we used a modified version of the acetone test,[41] in which a drop of acetone (~25 μL) is applied to the plantar surface of the hindpaw. Nocifensive behavior (lifting, flinching, and shaking of the paw) is then monitored for 1 minute. Motor coordination was evaluated using the rotarod test (standard Rotarod with a 1-inch diameter rotating cylinder; Harvard Apparatus, PanLab: Barcelona, Spain). Briefly, mice were first habituated on the still cylinder for 30 seconds and then trained at a constant speed (5 rpm) for 5 minutes. The next day (testing day), mice were placed on the rod at a constant rotation (4 rpm) for 10 seconds, followed by an acceleration from 4 to 40 rpm in 300 seconds. The latency to fall was recorded for each mouse (3 trials with a 15-minute intertrial interval). Because of size and weight, the experimenter performing the behavioral testing could not be blind to the different groups. As different experimenters tested male or female groups of mice, our analysis is only of young versus aged mice, not males versus females. All statistical analyses were performed with Prism (GraphPad: Boston, Massachusetts), and data are reported as mean ± Standard error of the mean. Student t-tests were used for single comparisons between 2 groups. Other data were analyzed using 2-way Analysis of varianc (ANOVA) followed by a post hoc Tukey's test.

Pruritogen-evoked Scratching

One day prior to testing, the mice were shaved at the nape of the neck. The following day, pruritogens (dissolved in saline) were injected subcutaneously (100 μL) into the neck: histamine (500 μg; Sigma: Burlington, Massachusetts) or chloroquine (100 μg; Sigma: Burlington, Massachusetts). We video-recorded the injected mice and counted scratching bouts that occurred during the first 30 minutes after injection.

Spared-nerve Injury Model of Neuropathic Pain

The spared nerve injury (SNI) model was produced as described previously.[42] Briefly, under isoflurane anesthesia, we ligated and transected 2 of the 3 branches of the sciatic nerve, sparing the tibial nerve. We tested the mechanical thresholds before (baseline), 7, and 21 days after injury. To test peripheral nerve injury-induced cold allodynia, we used the acetone drop test as described above, 7 days post-SNI. Postinjury heat thresholds were not tested as heat hypersensitivity is not commonly produced with this model.

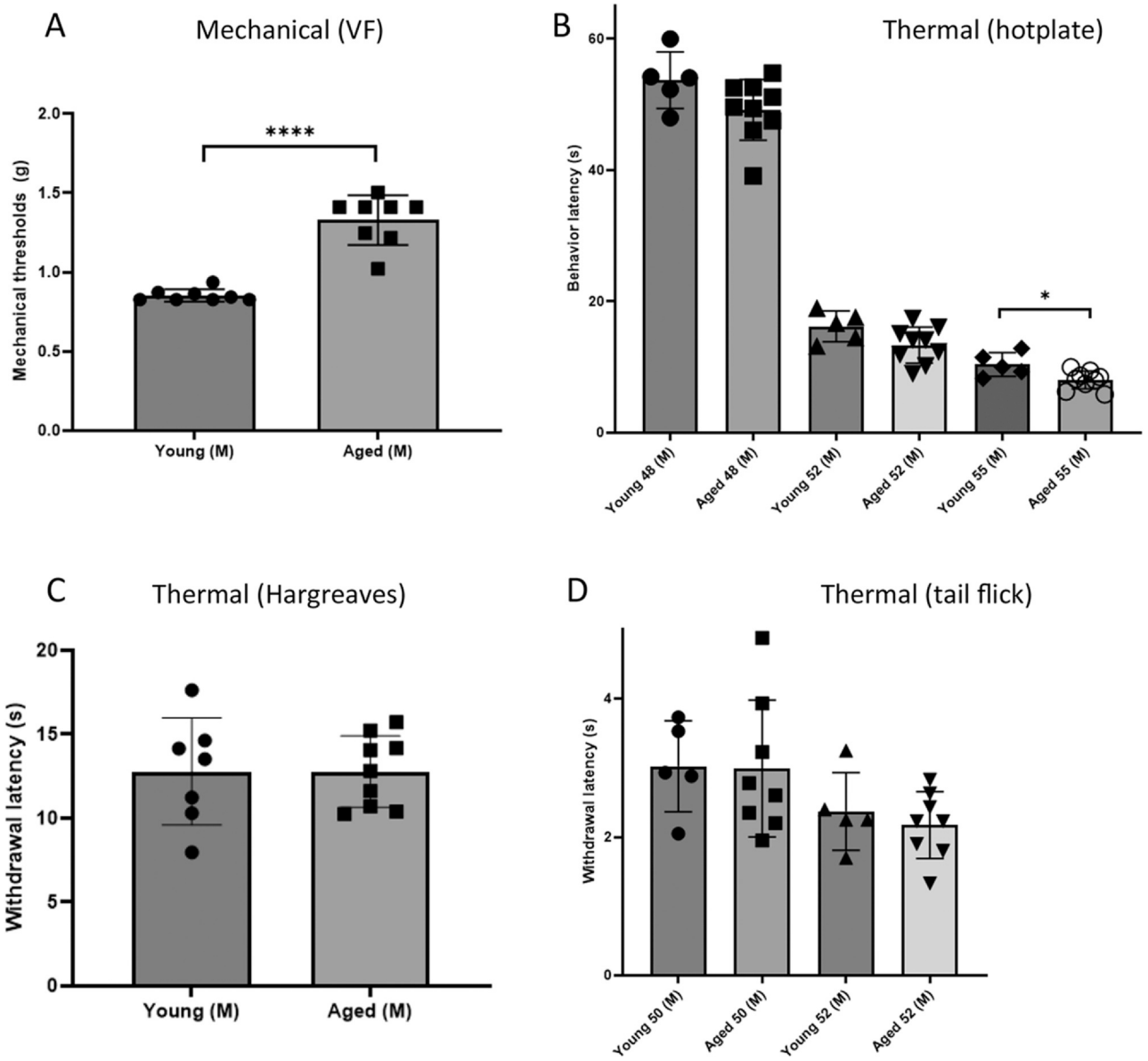

Figure 1. Aged male mice are hyporesponsive to mechanical but not thermal stimuli. (**A**) Compared to young mice (N = 8), aged male mice (N = 8) exhibited higher mechanical thresholds. (**B–D**) In contrast, young and aged male mice exhibited the same thresholds in response to thermal stimuli in the hotplate (**B**; N = 5 young and N = 9 aged mice), Hargreaves (**C**; N = 7 young and N = 9 aged mice) and tail flick (**D**; N = 5 young and N = 8 aged mice) tests. Data are presented as mean ± SEM; Student's t-test, *P < .05, ****P < .001. Abbreviation: SEM, Standard error of the mean.

Immunohistochemistry

Mice were anesthetized with an overdose of Avertin (250 mg/kg) and then perfused with 10 mL of saline, followed by 30 mL of 3.7% formaldehyde. Tissues were dissected out, postfixed, cryoprotected, and then sectioned on a cryostat. The tissue was immunostained as described previously.[43] Briefly, lumbar (L4 and L5), dorsal root ganglia (DRG; 14 μm), and spinal cord (25 μm) sections were preincubated for 30 minutes at room temperature in Tris-phosphate-buffered saline (0.5% Triton X-100, 10% bovine serum albumin, and 10% normal goat serum) and then immunostained overnight at room temperature in 10% Tris-phosphate-buffered saline containing the primary antibodies. After washing in PBS, sections were incubated for 1 hour with secondary antibody (Alexa 488- or 594-conjugated IgG; 1:700, Millipore: Burlington, Massachusetts), rinsed in PBS, mounted in Fluoromount-G (Southern Biotechnology, Birmingham, AL), and coverslipped. Antibodies used included activating transcriptional factor 3 (ATF3; rabbit, 1:2,000, Santa Cruz Biotechnology: Dallas, Texas), Iba1 (rabbit, 1:1,000, Wako: Madison, Wisconsin), Glial fibrillary acidic protein (GFAP) (mouse, 1:2,000, Sigma: Burlington, Massachusetts), and colony-stimulating factor 1 (CSF-1; goat, 1:1,000, R&D: Minneapolis, Minnesota). Fluorescent secondary antibodies were used at a 1:1,000 dilution.

Imaging

All images were taken on an LSM 700 confocal microscope (Zeiss: Oberkochen, Germany) equipped with 405-nm (5-mW fiber output), 488-nm (10-mW fiber output),

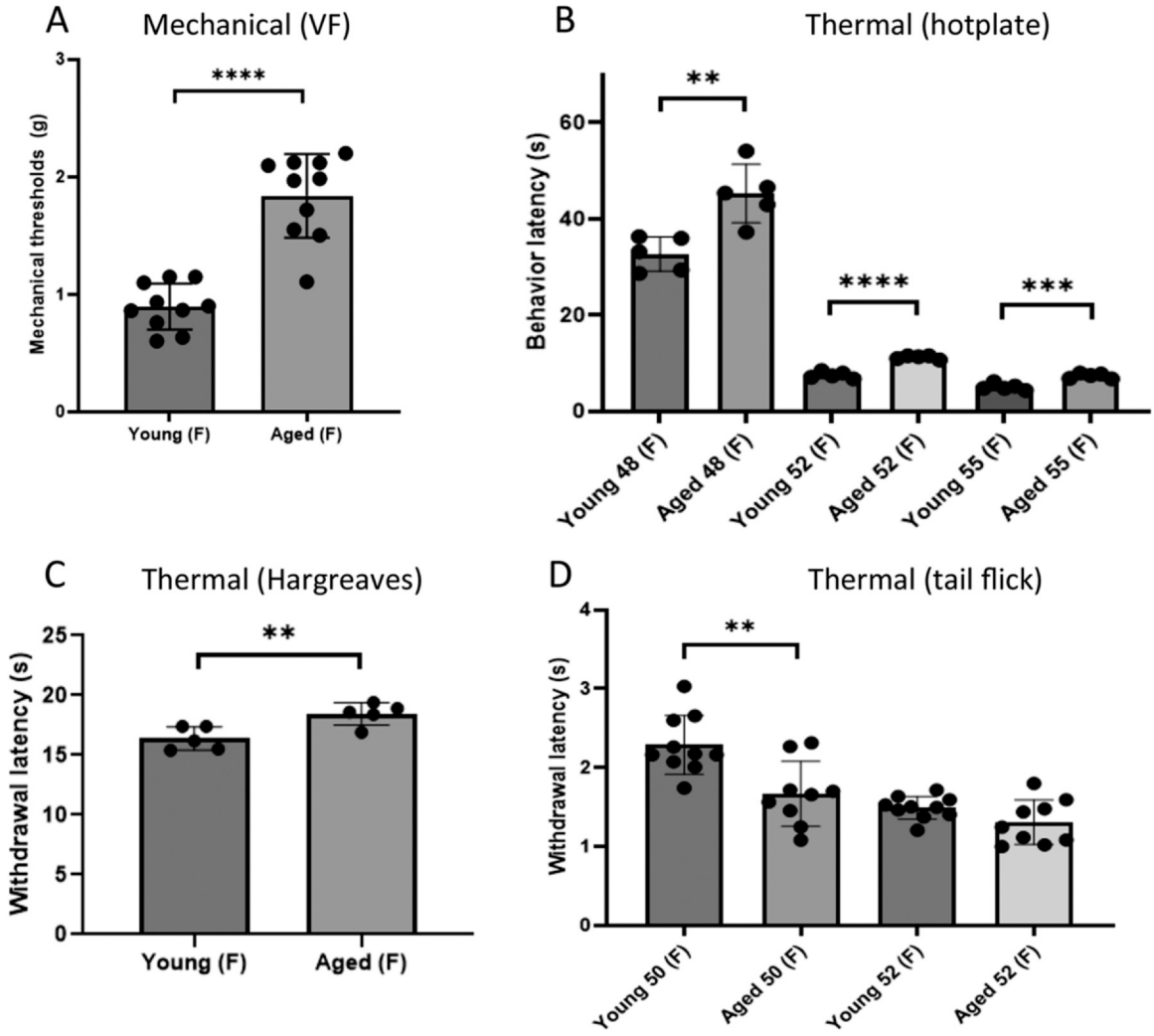

Figure 2. Aged female mice are hyporesponsive to both mechanical and thermal stimuli. Compared to young mice (N = 10), aged female mice (N = 10) exhibited higher mechanical thresholds (**A**) as well as longer response latencies in the hotplate (**B**; N = 5) and Hargreaves (**C**; N = 5) assays. In the tail flick test (**D**; N = 10 young and N = 9 aged mice), aged female mice showed shorter latencies at 50 but not 52 °C. Data are presented as mean ± SEM; Student's t-test, $^{**}P < .01$, $^{****}P < .001$. Abbreviation: SEM, Standard error of the mean.

555-nm (10-mW fiber output), and 639-nm (5-mW fiber output) diode lasers and a 20× Plan-Apochromat (20×/.8) objective (Zeiss: Oberkochen, Germany). Image acquisition was performed with ZEN 2010 (Zeiss: Oberkochen, Germany). Image dimensions were 1,024 × 1,024 pixels with an image depth of 12 bits. Two-times averaging was applied during image acquisition. To avoid saturation of single pixels, the laser power and gain were adjusted, and we used Fiji/ImageJ to control for brightness/contrast and assigning of artificial colors (look up table). The same imaging parameters and adjustments were used for all images within an experiment.

Results

Impaired Acute Mechanical Sensitivity in Aged Male Mice

For the behavioral analysis, we compared 6 months (young) and 22 to 24 months (aged) C57BL6 mice. Fig 1A shows that baseline mechanical thresholds (von Frey) of the aged male mice were significantly higher than those of young male mice. In contrast, only at the highest temperature tested in the hotplate assay (55 °C) did we record a significant decrease in baseline heat withdrawal thresholds (Fig. 1B–1D). At this temperature, the aged mice responded at shorter latency. Taken together, these findings indicate that aged male mice are hyporesponsive to mechanical and somewhat hyper-responsive to thermal (heat) stimuli at very high temperatures.

Sexual Dimorphism of Thermal Hyporesponsiveness in Aged Mice

To determine whether the age-related changes recorded in aged male mice are sexually dimorphic, we next tested the pain responsiveness of aged female mice across the same modalities. Fig 2A illustrates that, as for aged male mice, mechanical baseline thresholds were significantly higher in aged compared to young female mice. However, in contrast to the aged male mice, aged female mice also exhibited significantly higher noxious heat-induced withdrawal thresholds in the hotplate (Fig 2B) and Hargreaves (Fig 2C) assays. Unexpectedly, in the tail flick test, the aged female mice exhibited

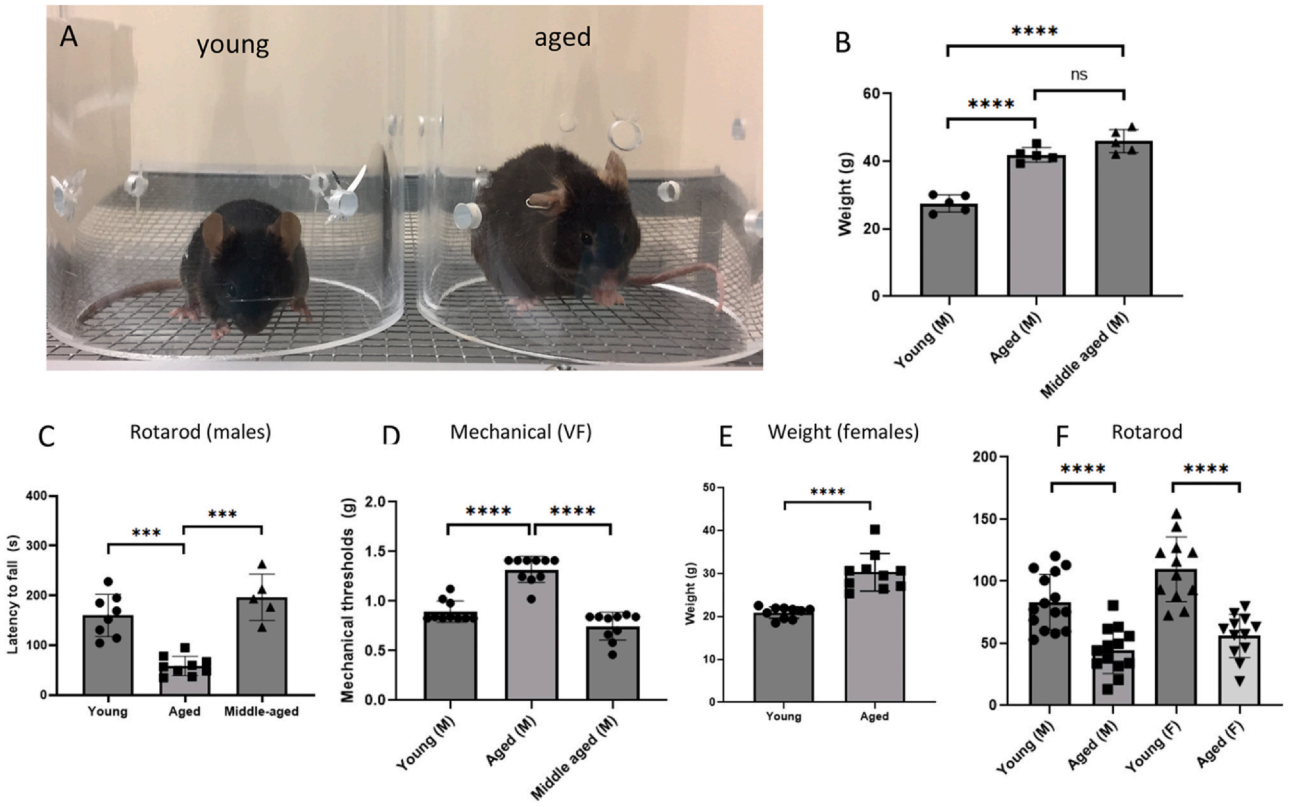

Figure 3. Age-related motor deficits are weight-independent. (**A–D**) Aged male mice are heavier (**A**, **B**) compared to young, lean mice (N = 5). Interestingly, although similar in weight (**B**), aged mice exhibited a shorter latency to fall in a rotarod test (**C**; N = 8 young and N = 9 aged mice; see also **F**) and higher mechanical thresholds (**D**; N = 10) than younger (middle-aged; N = 5), but overweight mice. (**E–F**) Aged female mice are also heavier (**E**; N = 10) and exhibit a shorter latency to fall off the rotarod (**F**; N = 12) than young female mice. Data are presented as mean ± SEM; 2-way ANOVA in **B** to **D** with post hoc Tukey's test; Student's t-test in **E** to **F**. ***P < .005, ****P < .001. Abbreviation: ns, not significant; SEM, Standard error of the mean; ANOVA, Analysis of variance.

decreased latency responses at 50 °C (Fig 2D). Taken together, we conclude that aged female mice are overall hyporesponsive in tests of acute pain to both mechanical and thermal stimuli.

Motor Impairment and Body Weight Gain in Aged Mice

Next, we tested whether the changes in baseline acute pain responses were a consequence of changes in the physical and/or motor characteristics of the aged mice. In these studies, in both young and aged mice, we recorded body weight and used the accelerated rotarod test to measure motor coordination. Fig 3 illustrates that aged male mice were significantly heavier (Fig. 3A and 3B; 2-way ANOVA $P < .0001$; F [2, 8] = 68.53) and showed a shorter latency to fall off the rotarod (Fig 3C; 2-way ANOVA $P < .0001$; F [2, 11] = 27.09) than did younger male mice. Interestingly, however, middle-aged (12 months) but also overweight mice had a motor performance comparable to that of the young but lean mice (Fig. 3B and 3C; 2-way ANOVA $P < .0001$; F[2, 18] = 94.68). Furthermore, despite being overweight, the middle-aged mice had mechanical withdrawal thresholds comparable to those of the young, lean mice (Fig 3D). In fact, both the young and middle-aged mice, regardless of weight, had lower mechanical withdrawal thresholds than the aged mice. Fig. 3E and 3F show that aged female mice were also heavier and showed a shorter latency to fall off the rotarod than younger female mice. Taken together, we conclude that weight gain had, at most, a limited and likely no contribution to the deficits observed in age-related motor coordination and nociceptive responsiveness.

Peripheral Nerve Injury Induces Comparable Ipsilateral Hypersensitivity in Aged and Young Mice

We next tested whether the changes in mechanical responsiveness recorded in aged mice persist in the setting of peripheral nerve injury. In these studies, we used the SNI model of neuropathic pain, which is manifest by profound mechanical allodynia and cold hypersensitivity. Because of significant differences in baseline mechanical thresholds in young versus aged mice, to better appreciate changes in the magnitude of the injury-induced allodynia, we normalized baseline thresholds. Fig. 4A and 4B illustrate that the mechanical thresholds of both young and aged mice, regardless of sex, decreased significantly 7 days after SNI. Interestingly, despite their higher baseline mechanical thresholds (Fig. 1 and 2), the aged mice developed ipsilateral

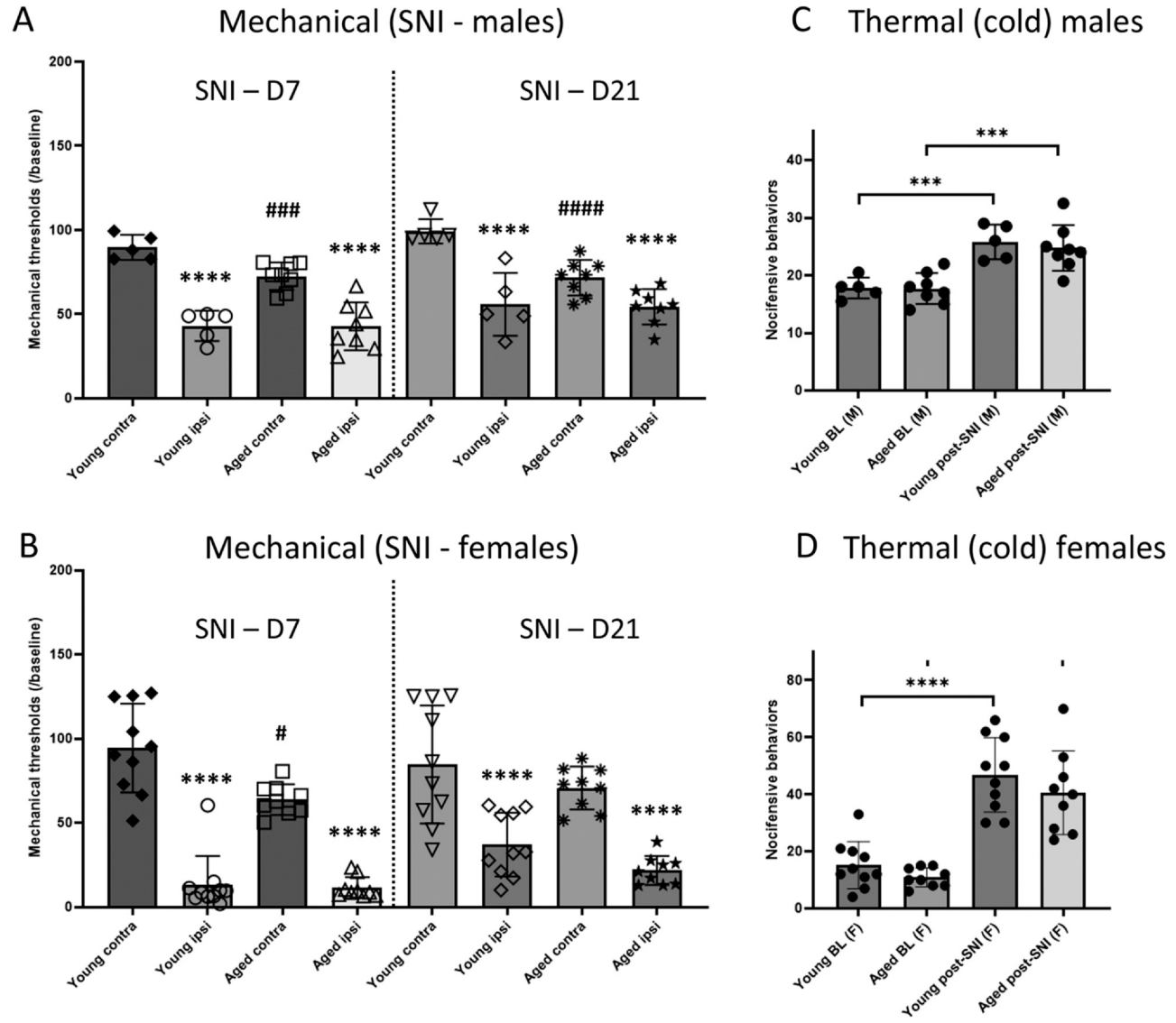

Figure 4. Aged mice develop normal ipsilateral mechanical and cold allodynia after peripheral nerve injury. (**A**) Seven and 21 days after SNI, young (N = 5) and aged (N = 8) male mice developed similar levels of mechanical allodynia ipsilateral to the injury. Interestingly, however, aged mice also developed mechanical hypersensitivity on the contralateral side, and the phenotype persisted for the entire observation time. (**B**) Young (N = 5) and aged (N = 9) male mice exhibited comparable levels of cold allodynia before or 7 days after SNI. (**C**) As in aged male mice, we recorded mechanical allodynia both ipsilateral and contralateral to the injury side in aged (N = 9) female mice, whereas young mice (N = 10) exhibited mechanical hypersensitivity only on the ipsilateral side. (**D**) Young (N = 10) and aged (N = 9) female mice exhibited similar responses to a hindpaw application of acetone before or 7 days after SNI. Data are presented as mean ± SEM; 2-way ANOVA in **A** and **C** with post hoc Tukey's test; Student's t-test in **B** and **D**, $^{\#}P < .05$, $^{\#\#\#/***}P < .005$, $^{\#\#\#\#/****}P < .001$; * Compared to baseline; # compared to the contralateral side. Abbreviation: SEM, Standard error of the mean, ANOVA, Analysis of variance.

mechanical allodynia at 7 and 21 days post nerve injury comparable to that recorded in the young mice. Furthermore, the aged but not the young mice also developed significant mechanical allodynia in the hindpaw contralateral to the injury. That hypersensitivity persisted for at least 21 days in aged males and for 7 days in aged females.

To measure cold allodynia that develops in the setting of neuropathic pain, we used the acetone drop test[44] and compared acetone-induced nocifensive behaviors (flinching, lifting, and shaking) in the aged and young mice. Fig 4 illustrates that both groups of mice showed comparable nocifensive behaviors (flinches and/or lifts) both before and 7 days after SNI, regardless of sex (Fig 4C males; Fig 4D females; 2-way ANOVA $P < .0001$; males: $F[11, 59] = 32.90$; females: $F[11, 93] = 28.15$). Taken together, these behavioral analyses indicate that measures of neuropathic pain develop normally in aged mice. However, the unexpected finding of contralateral mechanical hypersensitivity in the aged mice suggests that central nervous system changes secondary to unilateral nerve injury may be unique to the aged mice and may be relevant in the not uncommon observation of contralateral pain hypersensitivity in several clinical neuropathic pain conditions, including complex regional pain syndrome.[42]

Molecular and Anatomical Markers of Nerve Injury in Aged Mice

Consistent with a neuropathic pain phenotype developing normally in the aged mice, Fig 5 illustrates the concurrent expression of several hallmark markers of nerve injury in aged mice. Thus, ATF3 (red; Fig 5A) and CSF-1 (green; Fig 5A), two reliable markers of axotomized sensory neurons,[30,45] were both significantly induced in DRG neurons of the aged mice. As expected,[30] we also observed increases in markers of microglia and astrocyte activation in the spinal cord of the aged mice. Specifically, spinal cord immunoreactivity for both Iba1 (green, Fig 5B), a marker of microglia, and GFAP (red, Fig 5C), an astrocyte marker,

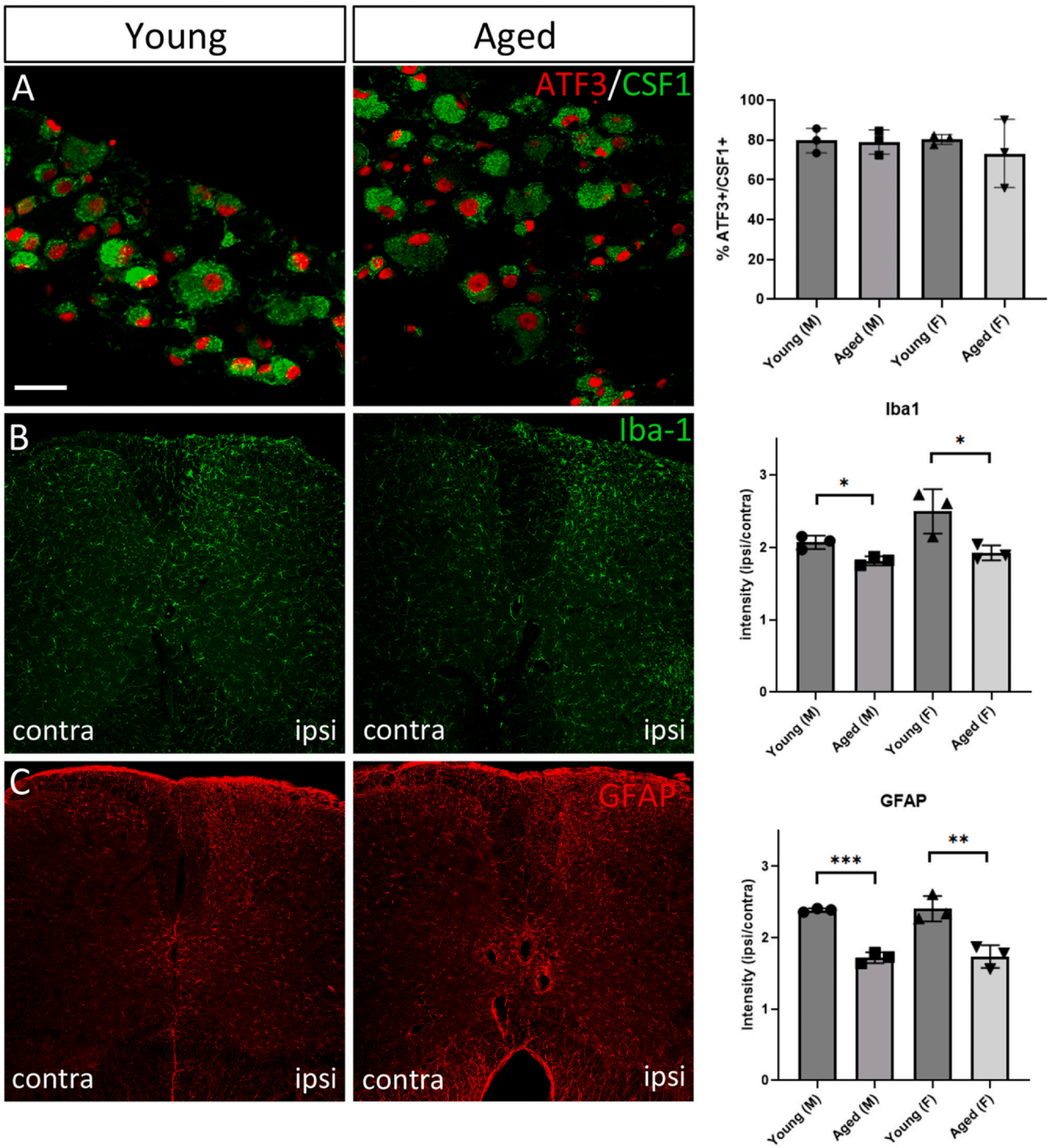

Figure 5. Molecular hallmarks of nerve injury in aged mice. (**A–C**) Dorsal root ganglia (**A**) and spinal cord (**B, C**) tissue from young and aged male and female mice (N = 3) were harvested and processed for immunohistochemistry. Expression of both ATF3 (red, **A**) and CSF1 (green, **A**) was induced in injured neurons in both groups of mice. In the spinal cord, peripheral nerve injury (SNI) increased immunoreactivity for Iba-1 (**B**) and GFAP (**C**), ipsilateral to the injury, in both groups of mice. Note that the ratio of ipsilateral to contralateral Iba-1 and GFAP immunoreactivity post injury decreased with age. Only representative pictures of immunostained tissue sections from male mice are shown. Data are presented as mean ± SEM; Student's t-test; **P* < .05, ***P* < .01, ****P* < .005. Scale bar: 100 μm. Abbreviation: GFAP, Glial fibrillary acidic protein; SEM, Standard error of the mean.

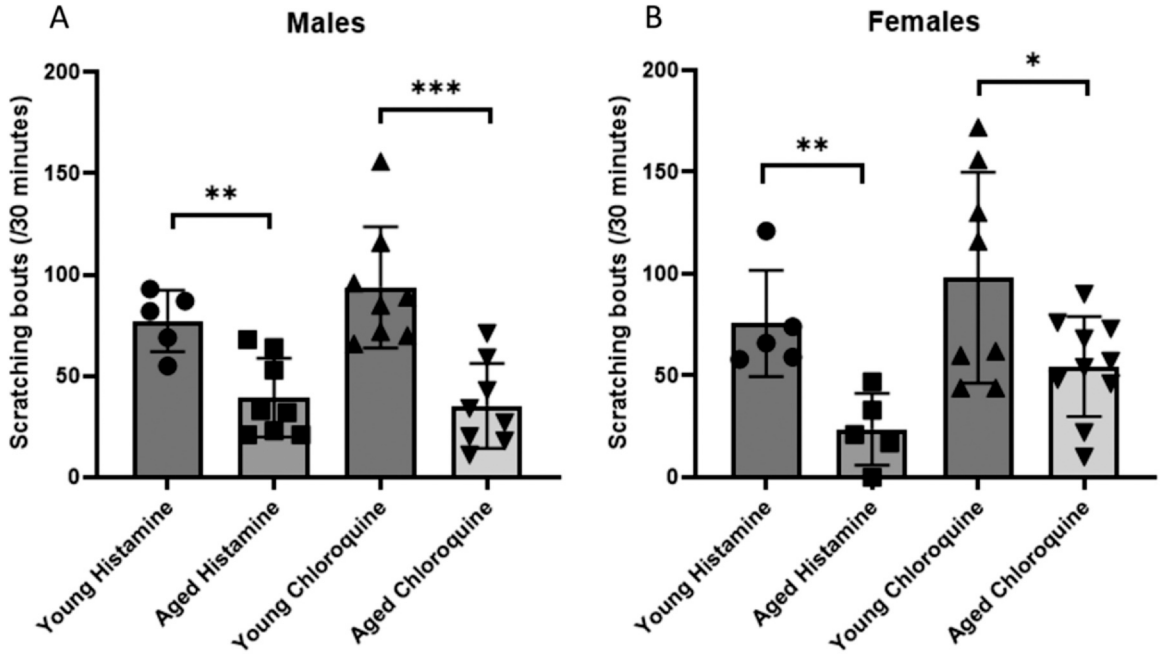

Figure 6. Pruritogens evoke less scratching in aged mice. A subcutaneous injection of histamine or chloroquine in the nape of the neck induced less scratching in both male (**A**; N = 5–8 young and N = 8 aged mice) and female (**B**; N = 5–8 young and N = 5–10 aged mice) aged mice compared to young mice. Data are presented as mean ± SEM; Student's t-test, $*P < .05$, $**P < .01$, $***P < .005$. Abbreviation: SEM, Standard error of the mean.

increased in the dorsal horn ipsilateral to the injury. Consistent with these spinal cord changes, we also found that transection of the infraorbital nerve in aged mice induced upregulation of Iba-1 in trigeminal nucleus caudalis, 7 days after the injury (data not shown). Interestingly, we found that the ratio of ipsilateral to contralateral microglial and GFAP expression in the spinal cord decreased with age, which we propose is consistent with the presence of both ipsilateral and contralateral mechanical hypersensitivities in the aged but not the young mice. Finally, and as expected, we found that the binding of isolectin B4 was significantly decreased in the dorsal horn of aged SNI mice (data not shown). Taken together, we conclude that the molecular mechanisms implicated in the initiation and maintenance of the neuropathic pain phenotype are intact (and perhaps even heightened) in the aged mice.

Decreased Pruritogen-evoked Scratching (Presumptive Itch) in Aged Mice

Finally, we examined whether aging influences the magnitude of scratching evoked by subcutaneous injection of pruritogens. In these studies, we injected the nape of the neck with histamine (500 μg) or chloroquine (100 μg) and video-recorded the number of scratching bouts in the following 30 minutes. Fig 6 shows that both male (Fig 6A) and female (Fig 6B) aged mice scratched significantly less than the younger mice in response to both histamine and chloroquine.

Conclusions

There are substantial discrepancies in the literature regarding age-related differences in pain sensitivity, both in humans and rodents, and therefore, we did not a priori predict that pain responsiveness would increase or decrease in the aged mice. However, as more animal studies have to date reported that pain behaviors increase, rather than decrease with age,[6] we were surprised to find that aged mice were overall hyporesponsive to acute noxious stimuli. What accounts for these discrepancies is unclear. Among the many factors that could contribute include testing methodology, rats versus mice, age and injury model studied. However, our preclinical findings are consistent with reports of decreased sensory perception and discrimination in the elderly, due in part to decreased nerve conduction,[46] a finding also reported in aged mice.[47]

As we measured nociceptive thresholds using reflex-based behavioral tests, it is also possible that the decreased responses that we observed were a consequence of sensory and/or motor impairments. For several reasons, we do not believe that this was the case. First, although aged male mice performed worse in the rotarod test, several tests involving withdrawal reflexes, which require motor function, did not differ. Specifically, the aged mice exhibited baseline thermal thresholds (males) as well as postinjury mechanical thresholds (males and females) comparable to those of young mice. The only exceptions recorded were a decreased response latency at the highest hotplate temperature tested in aged male mice and shorter withdrawal latencies in the tail flick assay at high temperatures in aged female mice. Second, although the aged mice were significantly heavier than the younger mice, we found that body weight was not a determining factor in pain responsiveness. In fact, young

but overweight mice exhibited similar mechanical thresholds as young but lean mice. On the other hand, we cannot exclude the possibility that the poor performance of aged mice in the rotarod test resulted from reduced motor coordination and/or neuromuscular strength[3] that were exacerbated by being overweight.

We also found that sex was not a determining factor for mechanical pain responsiveness; male and female aged mice exhibited similar changes. However, we did find an influence of sex on thermal responsiveness, with an aged female, but not aged male mice, also exhibiting significant deficits in acute thermal tests (Hargreaves and Hotplate). As a higher prevalence of acute and/or chronic pain conditions have been reported in females,[36,48] our finding of sex differences in pain responsiveness was not unexpected. However, as estrogen levels decrease with age,[49] one could have expected that estrogen influences on pain responsiveness would be mitigated in aged female mice. In fact, our results suggest that sex differences persist or may even be accentuated with age, independently of estrogen levels.

As the epidermal permeability barrier weakens with age, possibly due to structural changes,[50] the prevalence of chronic itch conditions also increases with age. Preclinical studies have reported similar findings. For example, dry skin pruritus is significantly worse in older mice, likely due to increased skin release of cytokines.[51] Although we have not examined the mice in models of chronic itch, it is of interest that we found that both male and female aged mice exhibited less scratching in response to acute pruritogens.

Perhaps more importantly, we found that aged mice developed and sustained mechanical hypersensitivity following nerve injury. This finding was corroborated in the patterns of expression of many pain-relevant neuroanatomical markers. Indeed, many hallmark markers of nerve injury were expressed at normal levels in aged mice, despite their having lower baseline responses to acute noxious stimuli in the absence of injury. Among the neurochemical findings were DRG induction of ATF3[45] and CSF1[30] in injured neurons as well as increased spinal cord microglial and astrocytic activation, not only ipsilateral but also contralateral to nerve injury.[52,53] Thus, many of the intracellular and molecular mechanisms implicated in the initiation and maintenance of the ipsilateral neuropathic pain phenotype appear to be induced normally in aged mice.

Undoubtedly there are many factors that increase the incidence of chronic pain in the elderly, notably disease (eg, osteoarthritis, cancer, chemotherapy, etc) as well as the greater vulnerability of the aged nervous system to external challenges. For several of these examples, dysfunction of the nervous system is likely a major contributor to the increased incidence of pain in the elderly, but here the dysfunction is secondary to some ongoing disease. For example, direct damage to the central nervous system (eg, post-stroke and multiple sclerosis) is a primary driver of chronic pain in the elderly. There is also preclinical evidence of primary nervous system dysfunction that likely contributes to increased pain in the elderly. For example, with age, brain microglia are more reactive to secondary insults,[54] which coupled with higher levels of stress hormones,[55,56] could enhance sensitivity to subthreshold challenges, despite lower baseline responsiveness. Furthermore, anxiety-like behaviors appear to increase with age,[57] which may negatively impact pain processing and/or facilitate the development of chronic pain syndromes. Indeed, high anxiety scores were predictive of an increased risk of pain onset in a rodent model of chronic inflammatory pain.[58] Aging also has measurable influences on brain function and plasticity,[59] and these, in turn, may result in long-term alterations in pain circuitry. Consistent with this hypothesis, we found that aged, but not young mice exhibited contralateral mechanical hypersensitivity that developed soon after the injury and lasted for several weeks, findings that we propose underlie the decreased ratio of post nerve injury ipsilateral to contralateral microglia and astrocyte activation. Undoubtedly synaptic changes that occur at both spinal and supraspinal levels[3] also contribute to the bilateral phenotypic changes in aged mice. Of interest, a recent study reported that dysfunction of the parabrachial and/or dorsomedial hypothalamic opioidergic system could lead to long-lasting bilateral mechanical allodynia in the mouse.[60] Future studies that assess the age-related changes that occur in the supraspinal regulation of spinal cord pain and itch processing will hopefully provide more insight into the differences that characterize aged and young animals.

Disclosures

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