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Changes in Spinal Serotonin Turnover Mediate Age-Related Differences in the Behavioral Manifestations of Peripheral Nerve Injury

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LOVELL, J. A., J. C. NOVAK, S. L. STUESSE, W. L. R. CRUCE AND T. CRISP. *Changes in spinal serotonin turnover mediate age-related differences in the behavioral manifestations of peripheral nerve injury.* PHARMACOL BIOCHEM BEHAV **66**(4) 873–878, 2000.—The Bennett and Xie model of peripheral nerve injury was used to study the effects of aging on the onset and progression of sciatic nerve ligation (SNL)-induced thermal hyperalgesia and tactile-evoked allodynia in young, mature, and aged Fischer 344 FBNF1 male rats (4–6, 14–16, and 24–26 months old, respectively). A plantar analgesia meter and calibrated von Frey pressure filaments were employed as the analgesiometric assays. In the absence of nerve injury, aged rats were found to be more sensitive than younger animals to noxious thermal stimuli. Following the SNL surgery, thermal hyperalgesia was observed in all three age groups within 3 days. On post-SNL day 35, the paw-withdrawal latency values of the young and mature animals returned to presurgical baseline levels, while the aged rats continued to exhibit thermal hyperalgesia. Tactile-evoked allodynia was apparent within 3 days following peripheral nerve injury in the oldest cohort, but was delayed in the younger animals. On post-SNL days 0 (control), 3, 21, and 35, young, mature, and aged rats were sacrificed and high-performance liquid chromatography and electrochemical detection (HPLC/ECD) methods were used for neurochemical analyses of spinal serotonin (5-HT), norepinephrine (NE), and 5-hydroxyindoleacetic acid (5-HIAA). Spinal 5-HT and NE levels were not significantly altered by the aging process, nor were they affected by peripheral nerve injury. However, spinal 5-HT turnover from the aged animals was greater than that detected in spinal tissue from the younger counterparts. Differences in spinal 5-HT turnover may contribute to age-related variability in spinal nociceptive processing.

Sciatic nerve Spinal cord Hyperalgesia Allodynia Serotonin Norepinephrine

CHRONIC disease processes (e.g., trigeminal neuralgia) and traumatic peripheral nerve injury can cause chronic neuropathic pain disorders. Examples of the types of peripheral neuropathies that are prevalent in senescent individuals include diabetic neuropathy and postherpetic neuralgia (9,15). The prevailing symptoms of chronic nerve pain are hyperalgesia (an exaggerated response to a normally painful stimulus) and tactile-evoked allodynia (a painful response to a normally innocuous stimulus). These devastating symptoms can persist for years, and are often unresponsive to conventional analgesics in clinical practice (1,2,6).

Descending serotonergic (5-HT) and noradrenergic (NE) fibers comprise what is known as the descending pain-inhibitory system, which functions to curtail nociceptive input to the spinal cord (3). Satoh and Omote (21) reported that tissue levels of NE and 5-HT increased significantly in the lumbar dorsal horn of rats 7 days after the induction of peripheral nerve injury. Moreover, the intrathecal (IT) administration of

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5-HT and NE receptor antagonists (methysergide and yohimbine, respectively) enhanced the thermal hyperalgesic responses of nerve-injured animals (21). These findings confirm the role of descending serotonergic and noradrenergic neuronal pathways in modulating nerve injury-induced nociceptive input to the spinal cord.

Both 5-HT and NE are degraded by the enzyme monoamine oxidase (MAO), and MAO activity reportedly increases as a function of age (7,19,20). Previous studies in this laboratory have demonstrated that spinal 5-HT and NE levels decrease as age increases (16). Although it is not yet known if the aging process alters the pain-inhibitory properties of the descending pain-inhibitory system, it seems reasonable to speculate that an age-related decrease in spinal 5-HT and NE levels would diminish the pain-inhibitory efficacy of the descending analgesic system. The present study was designed to assess age-related differences in the onset and maintenance of chronic nerve pain, and to ascertain if differences in nociceptive processing are due to age-dependent changes in spinal biogenic amine levels. These findings will be useful for providing more effective treatments for patients of all ages who are afflicted with chronic pain of neuropathic origin.

METHOD

Animals

Young, mature, and aged male Fischer 344 FBNF1 rats (4–6, 14–16, and 24–26 months old, respectively) were used in all experiments (n = at least six rats/age group). All animals were housed individually in clear plastic cages containing at least 4 cm of shredded aspen bedding covering the floor of the cage. Food and water were available ad lib. The behavioral and neurochemical experiments described herein were fully approved by the Institutional Animal Care and Use Committee at Northeastern Ohio Universities College of Medicine.

Sciatic Nerve Ligation (SNL) Surgery

The Bennett and Xie (5) model of peripheral nerve injury was used to study the effects of aging on the onset and progression of sciatic nerve ligation (SNL)-induced thermal hyperalgesia and tactile-evoked allodynia in young, mature, and aged rats. Prior to the SNL surgery, rats were weighed and anesthetized with pentobarbital 45 mg/kg, IP. The left sciatic nerve was exposed and, proximal to the trifurcation, approximately 7 mm of nerve was freed of adhering tissue. Four 4-0 chromic gut sutures were loosely tied around the nerve at intervals of approximately 1 mm. Ligatures were tied loosely enough so that, upon visual inspection, blood flow was not obstructed. The surgical incision was sutured and postsurgical recuperation was monitored daily.

Analgesiometric Assays

Paw-withdrawal latency (PWL) values. A plantar analgesic meter was used to record pre- and post-SNL paw withdrawal latency (PWL) values from the left and right hind paw of each rat. These values were used to measure age-related differences in the onset and progression of nerve injury-induced thermal hyperalgesia. In these studies, PWL was defined as the time of initial exposure of a thermal stimulus to the plantar surface of the hind paw to the time of withdrawal of the paw from the heat source. A 20-s limit of heat exposure was imposed to preclude tissue damage to the paw. Four presurgical PWL measurements were recorded from the left and right hind paws at 10-min intervals, and an average of the last three was calculated and defined as the presurgical PWL (day 0 control). To quantify the development of thermal hyperalgesia, difference scores were computed for each rat by subtracting the PWL of the unligated hind paw from the PWL of the ligated hind paw. Thermal hyperalgesia was manifested as a negative difference score. To investigate age-related differences in the development and progression of SNL-induced thermal hyperalgesia, postsurgical PWL measurements were collected on postligation days 3, 21, and 35 between 0800 h and noon. On each test day, three PWL values were recorded from the left and right hind paw at 10-min intervals, and an average of the last two measurements was calculated. To curtail experimental bias, experimenters performing the behavioral assessments were blind to the age of the animals.

Paw-withdrawal threshold (PWT) values. Calibrated von Frey filaments were used to obtain pre- and postligation paw withdrawal threshold (PWT) values from young, mature, and aged rats. The von Frey methodology is effective for measuring the onset and progression of tactile-evoked allodynia by assessing the sensitivity of the skin to tactile stimulation. Animals were placed under a transparent dome on a plastic mesh floor, and increasing filament strength was applied sequentially to the plantar surface of the ligated and unligated hind paw at 10-min intervals. Pre- and post-SNL PWT values were defined as the minimum gram strength eliciting two sequential responses (lifting of paw). Postsurgical PWT measurements were collected on post-SNL days 3, 21, and 35 between 0800 h and noon.

Tissue Preparation for HPLC/ECD Analyses

Rats were sacrificed via decapitation on post-SNL days 0, 3, 21, or 35, and the spinal cords were quickly removed and frozen at −75°C. Lumbar sections were divided into dorsal and ventral halves and weighed, and care was taken to ensure that the spinal tissue remained frozen throughout the cutting procedure. On the day of neurochemical analysis, samples were homogenized in 1 ml of 0.1 N perchloric acid/0.1 mM EDTA solution with an ultrasonicator (17). The homogenates were centrifuged for 15 min at 4°C with a microcentrifuge, filtered through 0.45-μm syringe filters, and 20 μl of the supernatant was used for analysis via HPLC and electrochemical detection. The mobile phase buffer (pH 3.0) contained 0.15 M monochloroacetic acid, 0.1 mM EDTA, 3.5% acetonitrile and 1 mM octyl sodium sulfate as the ion-pairing agent. A 3-μm particle reverse-phase catecholamine cartridge column (Phase II ODS, BAS) was used, and the applied voltage was +0.7 V vs. a Ag/AgCl reference electrode. The integrator was calibrated daily by injecting 200 picogram (pg) standards (5-HT, NE, and 5-HIAA) prior to running the samples and after analysis of every fourth sample. The standards were made fresh daily.

Statistical Analysis

Presurgical PWL and PWT values were statistically analyzed with a one-way repeated ANOVA to determine if significant age-related differences exist prior to the SNL surgery. A two-way repeated-measures ANOVA was used to detect postsurgical differences in thermal hyperalgesia and tactile-evoked allodynia as a function of age. Neurochemical results are expressed as pg biogenic amine/mg tissue wet weight. Statistical analysis was performed using a two-way ANOVA and by linear regression (Systat). An alpha level of 0.05 was considered statistically significant.

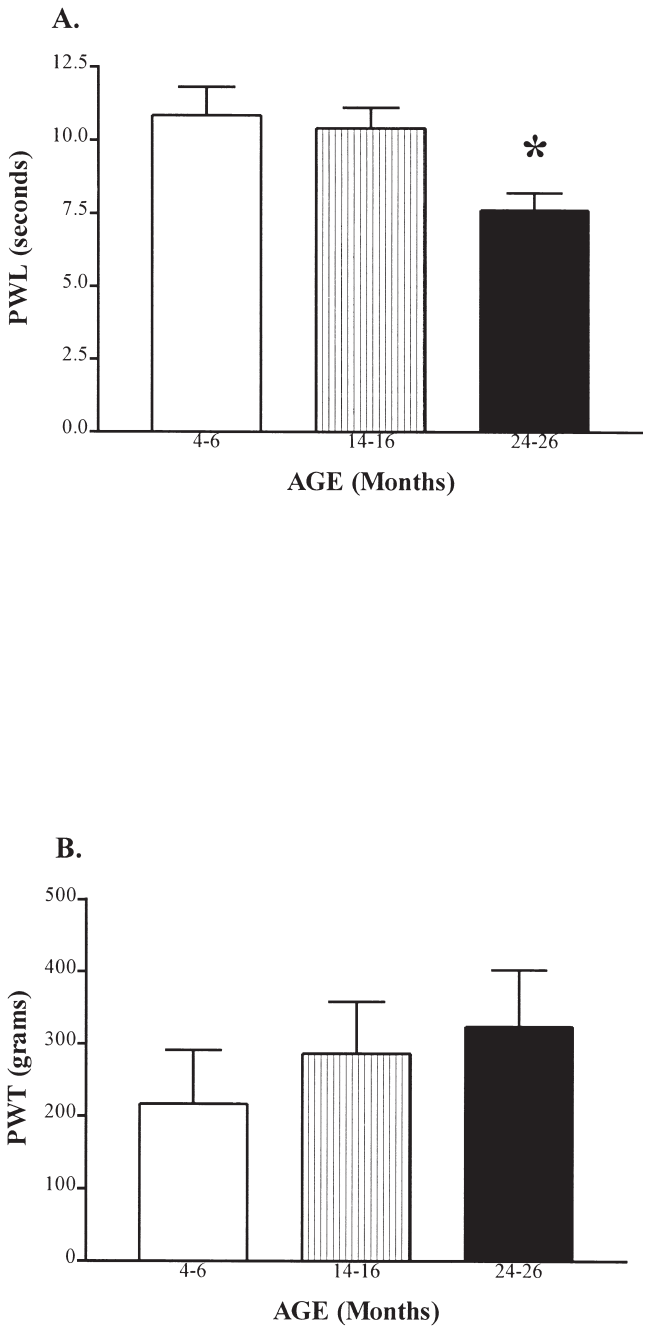


FIG. 1. Presurgical paw withdrawal latency (PWL) responses to a thermal nociceptive stimulus (A) and paw withdrawal threshold (PWT) responses to a tactile stimulus (B) from the left hind paw of unligated young (4–6 months old), mature (14–16 months old), and aged (24–26 months old) rats. Data represent the mean ± SEM of n = at least 6 rats/age group. *$p \leq 0.05$ vs. 4–6- and 14–16-month-old rats.

RESULTS

In the absence of nerve injury (Fig. 1A), the aged animals responded significantly faster than the younger cohorts to the thermal nociceptive stimulus. Conversely, no significant differences were detected in threshold responses to tactile stimuli across the three age groups (Fig. 1B).

To verify the development of thermal hyperalgesia following the induction of peripheral nerve injury, difference scores were calculated for each rat by subtracting the PWL of the unligated hind paw from the PWL of the ligated hind paw. In unligated (control) animals, difference scores were not significantly different across the three age groups (e.g., 4–6-month-old rats = 0.0 ± 0.6 s; 14–16-month-old rats = 1.1 ± 0.7 s; 24–26-month-old rats = 0.0 ± 1.4 s). As depicted in Fig. 2A, all age groups developed robust thermal hyperalgesic responses which were of similar magnitude by postligation day 3. Hyperalgesia to the thermal stimulus was manifested as a negative difference score, which demonstrated that the left (ligated) hind paw responded significantly faster than the right (control) hind paw (Fig. 2A).

By postligation day 21, PWL difference scores in the youngest cohort of animals were not significantly different from presurgical control values, indicating the onset of recovery from SNL-induced thermal hyperalgesia. Conversely, the mature and aged cohorts continued to display difference scores that were significantly less than the age-matched control values, suggesting the persistence of thermal hyperalgesia in the older age groups (Fig. 2B).

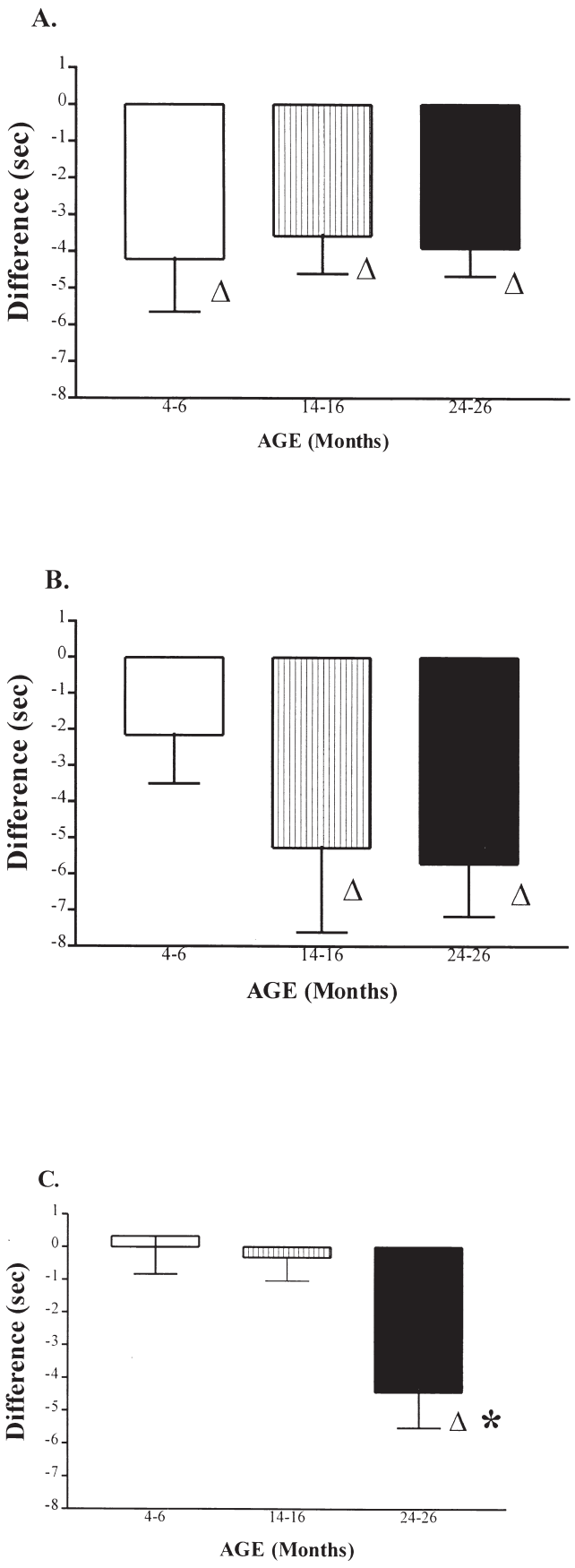
On post-SNL day 35, the difference scores obtained from the 4–6- and 14–16-month-old rats were not significantly different from pre-SNL age-matched controls, whereas the aged animals continued to exhibit robust hyperalgesic responses (Fig. 2C). These findings demonstrate age-related differences in the recovery from peripheral nerve injury-induced thermal hyperalgesia in young, mature, and aged rats.

The von Frey method was used to obtain pre- and postligation paw withdrawal threshold (PWT) values from young, mature, and aged rats to ascertain the effects of aging on SNL-induced tactile-evoked allodynia. On post-SNL day 3, PWT values from the aged animals were significantly less than presurgical age-matched controls and from the tactile responses of the young and mature cohorts (Fig. 3A). Thus, 3 days after nerve injury, tactile allodynia was exhibited only by the aged cohort. By postligation day 21, the mature and aged rats developed robust tactile allodynic responses (Fig. 3B) while SNL-induced allodynia was not evident in the youngest group of animals until postsurgical day 35 (Fig. 3C). Also depicted in Fig. 3C is the observation that significant tactile-evoked allodynic responses were evident in all age groups through postligation day 35.

Our neurochemical findings revealed no consistent age-related relationship between the onset and duration of the behavioral manifestations of neuropathic pain and changes in spinal biogenic amine levels. As shown in Table 1, no significant differences due to aging were detected in pre- and post-SNL 5-HT and NE levels in the dorsal half of the lumbar spinal cord. However, linear regression analyses revealed that 5-HT turnover was significantly elevated in the spinal tissue from unligated and ligated 24–26-month-old animals compared to the younger counterparts.

DISCUSSION

The experiments comprising this study were designed to assess age-related differences in the development and progression of chronic nerve pain and to determine if changes in nociceptive processing were attributable to age-dependent differences in spinal biogenic amine levels. Our findings revealed that, in the absence of nerve injury, senescent animals exhibited a heightened sensitivity to the thermal nociceptive stimulus. These findings are in accord with our earlier behav-

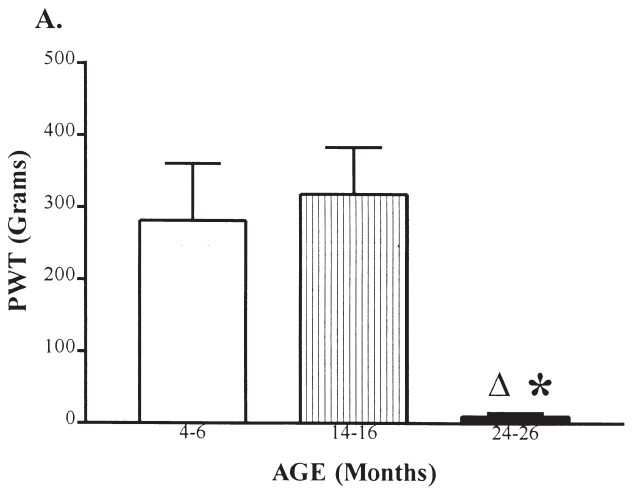


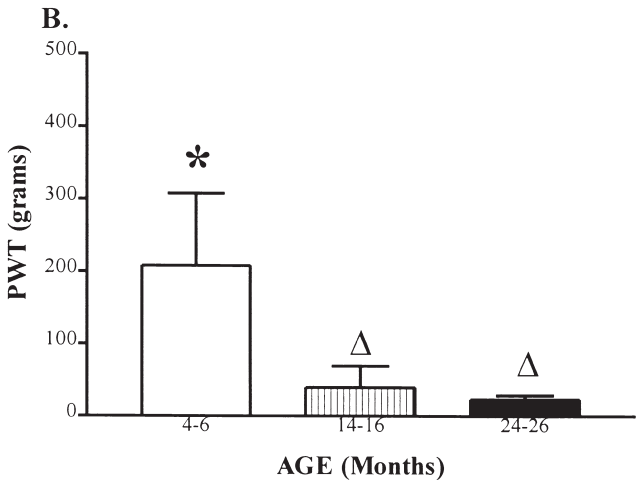
ioral work showing that aged rats responded significantly faster to an acute thermal nociceptive stimulus than did their younger counterparts (13,18). However, no significant differences were detected in the threshold responses of unligated animals to tactile-evoked stimuli across the three age groups, suggesting that the specific type of stimulus being employed (e.g., thermal, mechanical or chemical) may differentially affect the response parameters of different age cohorts.

The present results also revealed that the onset and duration of nerve injury-induced thermal hyperalgesia and tactile-evoked allodynia differed as a function of age. In the thermal hyperalgesic studies, all three age groups displayed robust hyperalgesic responses to a thermal nociceptive stimulus by postligation day 3, but the effects in aged animals were longer lasting (e.g., difference scores from the young and mature animals returned to age-matched control values by post-SNL day 35, whereas the aged animals continued to exhibit robust hyperalgesic responses throughout the 35 day duration of the experiments; Fig. 2C). Although these findings suggest that the aging process somehow diminishes the potential for recovery from peripheral nerve injury in aged animals, the steep mortality curve of rats older than 27–28 months precludes an assessment of the potential pattern of recovery in the oldest cohort. It is, however, entirely possible that the aged animals would have eventually recovered if the behavioral experiments had been carried out longer than 35 days postligation.

Descending serotonergic projections from the brain stem to the spinal cord play a mediatory role in spinal antinociceptive processing (3). Recent neurochemical studies in this laboratory demonstrated that spinal levels of 5-HT and NE decrease as a function of age in rats (16). An age-related impairment of the biogenic amine neurotransmitters comprising the descending pain-inhibitory system could explain the heightened sensitivity of our unligated senescent animals to thermal nociception. However, no age-related differences in the content of 5-HT or NE were detected in lumbar spinal tissue from pre- or postligated young, mature, or aged rats in this study. Perhaps the disparate neurochemical findings of Ko et al. (16) and those of the present study are attributable to differences in the strain of rat being used. Fischer 344 rats were used in our previous investigations (16), whereas a hybrid strain of Fischer rats (FBNF1) was used in the experiments comprising this series of investigations. Because different strains of rats are often used in preclinical investigations of chronic nociceptive processing (5,6,14,17,21), it is crucial to clarify the potential influence of animal strain on the perception and treatment of pain. A recent report of strain differences in peripheral nerve injury-induced thermal hyperalgesia (17) supports the notion that spinal neurochemistry may differ as a function of strain. Further, although Davies et al. (14) observed concomitant reductions in spinal biogenic amine content and thermal hyperalgesia following lesions of the dorsolateral funiculus in rats (Alderley Park Strain, ICI), these investigators were unable to establish a clear correlation between the magnitude of loss of 5-HT and NE and the degree of thermal hyperalgesia. Thus, despite the well-established pain modulatory role of the biogenic amine neu-

FIG. 2. Difference scores from young, mature and aged rats on postligation days (A) 3, (B) 21, and (C) 35. Data represent mean difference scores ± SEM from $n = 6$ rats/age group. $\Delta p \leq 0.05$ vs. pre-SNL age-matched controls, $*p \leq 0.05$ vs. 4–6- and 14–16-month-old animals.





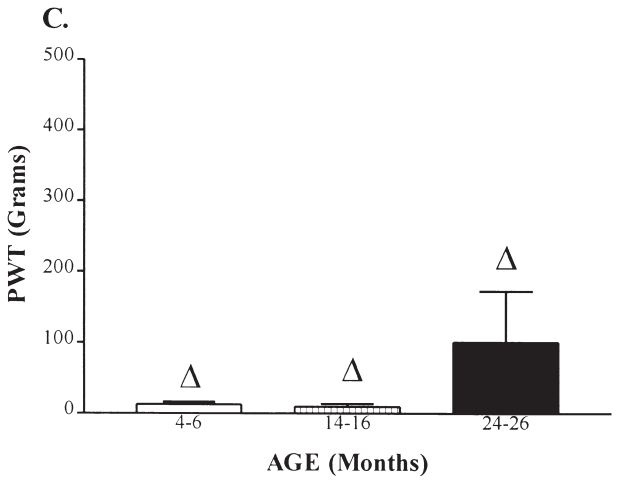


TABLE 1

SEROTONIN (5-HT) AND NOREPINEPHRINE (NE) CONTENT (PG/MG WET TISSUE WEIGHT) AND 5-HT TURNOVER FROM THE DORSAL HALF OF THE LUMBAR SPINAL CORD OF UNLIGATED VERSUS LIGATED YOUNG, MATURE, AND AGED RATS.

Days Post-SNL	AGE (Months)		
5-HT (pg/mg wet Tissue Weight)	4–6	14–16	24–26
0 (pre-SNL control)	17.4 ± 2.2	14.9 ± 2.8	26.9 ± 3.2
3	21.9 ± 3.5	24.3 ± 6.4	27.0 ± 5.9
21	17.3 ± 3.7	14.8 ± 1.9	20.0 ± 3.2
35	23.1 ± 5.2	15.4 ± 2.1	18.4 ± 3.4
NE (pg/mg wet Tissue Weight)			
0 (pre-SNL control)	6.5 ± 0.6	5.5 ± 0.5	5.4 ± 0.7
3	7.4 ± 0.8	6.0 ± 0.5	5.7 ± 0.7
21	5.8 ± 0.3	5.5 ± 0.4	7.2 ± 0.7
35	6.0 ± 0.7	5.7 ± 0.6	5.7 ± 0.8
5-HT Turnover (5-HT/5-HIAA)	0.8 ± 0.1	1.0 ± 0.1	1.2 ± 0.2*

*$p \leq 0.05$ vs. the 4–6-month-old cohort.

rotransmitters, the underlying spinal mechanisms have yet to be fully elucidated.

In concordance with earlier reports (23), linear regression analyses of our neurochemical data revealed that 5-HT turnover was significantly elevated in spinal tissue from 24–26-month-old animals, suggesting that the structural integrity of bulbospinal serotonergic nerves remains intact with advanced age. However, whether the pain-inhibitory properties of the descending biogenic amine pathways change as a function of age remains to be determined. As previously reported (13), the oldest cohort of rats exhibited a heightened sensitivity to thermal nociception, and we hypothesized that the antinociceptive properties of 5-HT–containing bulbospinal projections decline with advanced age. Monoamine oxidase (MAO) is responsible for the enzymatic degradation of 5-HT, and MAO activity increases as a function of age (7,19,20). The enhanced sensitivity of aged rats to thermal stimuli, as well as the age-related increase in spinal 5-HT turnover, could be attributable to an age-related increase in the metabolism of spinal 5-HT. Further, the spinal antinociceptive efficacy of 5-HT could potentially be diminished by age-dependent alterations in receptor number, affinity or postreceptor transduction mechanisms.

It is well established that enzymatic degradation of the biogenic amine neurotransmitters via MAO generates the production of free radicals, which can cause oxidative stress responses (10). Free radical-induced oxidation is thought to play an important mediatory role in several neurodegenerative diseases common in the senescent population (4,11–13). An age-related increase in spinal MAO activity could conceivably enhance the susceptibility of pain-modulatory neurons in the spinal cord to oxidative stress. Such a scenario would explain our behavioral findings that aged animals have a heightened sensitivity to nociceptive stimuli. Perhaps an augmented oxidative stress response in the aged cohort also explains the lack of recovery of the senescent animals from SNL-induced thermal hyperalgesia.

FIG. 3. Paw withdrawal threshold (PWT) values from young, mature, and aged rats on postligation days (A) 3, (B) 21, and (C) 35. Data represent mean PWT values ± SEM from $n = 6$ rats/age group. $\Delta p < 0.05$ vs. pre-SNL age-matched controls, $*p \leq 0.05$ vs. the other two age cohorts.

Studies designed to investigate this possibility are currently underway in this laboratory.

Glutamate is an endogenous excitatory amino acid (EAA) that interacts with NMDA and AMPA receptor sites in the spinal cord to mediate chronic pain of neuropathic origin (8). The synaptic uptake of glutamate is diminished in aged animals, potentially increasing the susceptibility of senescent rats to EAA-induced excitotoxicity (22). Further, the enhanced synaptic activity of spinal glutamate in aged rats may potentially damage bulbospinal biogenic amine-containing nerve terminals in the dorsal horn. As suggested, an age-dependent attenuation of the antinociceptive properties of the descending pain-inhibitory system may be behaviorally manifested as an enhanced thermal hyperalgesic response to peripheral nerve injury. Moreover, because the excitotoxic actions of glutamate in the CNS are irreversible, aged animals would be less likely to recover from peripheral nerve injury.

In summary, animal studies investigating age-related differences in the symptomatology of peripheral nerve injury are scarce. The present results demonstrate that the aging process differentially affects chronic nociceptive processing and underscore the need for further investigation to develop more effective treatment strategies for patients of all ages.

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