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Involvement of Dorsal Column Nucleus Neurons in Nociceptive Transmission in Aged Rats

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Kitagawa, Junichi, Yoshiyuki Tsuboi, Akiko Ogawa, Ke Ren, Suzuro Hitomi, Kimiko Saitoh, Osamu Takahashi, Yuji Masuda, Toshiyuki Harada, Naoki Hanzawa, Kenro Kanda, and Koichi Iwata. Involvement of dorsal column nucleus neurons in nociceptive transmission in aged rats. *J Neurophysiol* 94: 4178–4187, 2005; doi:10.1152/jn.00243.2005. To clarify the functional role of the dorsal column nucleus (DCN) in nociception in rats with advancing age, single neuronal activity and substance P–like immunoreactivity (SP-LI) of the gracile nucleus (GN) were studied in aged rats (29 to 34 mo old) and adult rats (9 to 12 mo old). A total of 122 neurons [aged: 34 wide-dynamic-range (WDR), two nociceptive-specific (NS), and 32 low-threshold mechanical (LTM) neurons; adult: 22 WDR and 32 LTM neurons] were recorded from GN. For WDR neurons, the latency to antidromic activation of the ventral posterior lateral nucleus of the thalamus showed no difference between the aged and adult rats. Sciatic nerve stimulation with C-fiber intensity induced responses of GN with significantly longer latency in aged rats than in adults, whereas there was no difference in the response latency to A-fiber intensity stimulation. Background activity and afterdischarges were significantly higher in the aged rats than those in the adult rats. Responses to noxious mechanical and thermal stimuli were significantly greater in the aged rats during application of graded stimuli. There were no significant differences in responses to nonnoxious mechanical stimulus, mechanical response threshold, and the size of the receptive fields between neurons in the aged and adult rats. The area occupied by SP-LI fibers in the GN and the size of SP-LI dorsal root ganglia neurons were significantly larger in aged rats than in adults. The present findings suggest that the hyperexcitability of GN neurons *could* be involved in abnormal noxious pain sensations with advancing age.

INTRODUCTION

The effects of aging on pain have been studied in human subjects using psychophysical methods. Most studies report that the pain threshold is significantly increased in the aged subjects (Harkins et al. 1986, 1988; Schludermann and Zubek 1962), whereas some studies showed no change (Kenshalo 1986). In animal studies, it is reported that there is significant modification of neurochemical substances related to nociception in the spinal dorsal horn (DH), such as substance P (SP) and serotonin (Charlton and Helke 1986; Laporte et al.1996). The neurochemical modulation in the spinal DH may be associated with changes in DH neuronal activity. It has been recently reported that the DH neuronal activity is significantly greater in aged rats after noxious chemical stimulation of the hind paw (Zheng et al. 2004). The modulation of neuronal activity in the DH would be related to nociceptive reflex or nocifensive behavior. However, the functional relationship between the DH neuronal activity and nocifensive behavior with advancing age is still unknown. Some animal studies have examined nocifensive behavior in aged rats and found that nocifensive behavior was decreased after formalin injection into the hind paw (Gagliese and Melzack 1999, 2000). Thus there are discrepancies in neuronal activity and behavioral data between aged and young rats.

Recently we investigated nociceptive neurons in the spinal DH in aged rats (Iwata et al. 2002). The spontaneous activities and afterdischarges following mechanical stimulation were higher in aged rats than in adult rats. Response to heat stimulation was also higher in aged rats. Immunohistochemical analysis showed that serotonin- and noradrenalin-like immunoreactive (LI) fibers were significantly decreased in the spinal DH, and spinal block did not affect the neuronal activity in the DH neurons in aged rats. Thus the descending pain inhibitory system appears to be impaired in aged rats (Iwata et al. 1995, 2002; Kanda et al. 2001), where the deficit of the descending modulation system is responsible for the increased activity of the nociceptive neurons in the spinal DH. These data, together with the above-stated discrepancy of the data among human psychophysical experiments, may be the result of a complex effect of aging on pain pathways. Neuronal death has been observed in many parts of the CNS in aged human and animal subjects (Bergman and Ulfhake 1998; Bergman et al. 1996, 1999; Brena and Bonica 1970; Crisp et al. 1994; Fujisawa et al. 1978; Goicoechea et al. 1997; Ko et al. 1997). Findings suggesting the existence of compensatory reactions to degenerative changes have been reported (Bergman et al. 1996, 1999; Crisp et al. 1994; Fujisawa et al. 1978; Goicoechea et al.

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1997; Ko et al. 1997). Thus it is reasonable to hypothesize that reorganization arising from neuronal death and compensatory reactions are taking place in the pain system during the aging process.

There are two known major sensory pathways for ascending sensory information to the higher CNS: the spinothalamic and dorsal-column systems. The spinothalamic system is one of the major pain-transmission pathways (Chung et al. 1979; Kenshalo et al. 1979, 1982; Willis and Coggeshall 1991), whereas the dorsal-column system is believed to be mainly involved in relaying innocuous sensory information (Cliffer et al. 1992; Ferrington et al. 1988; Giesler and Cliffer 1985). However, it has been reported that dorsal-column nucleus neurons change their response properties after peripheral nerve damage (Kondo et al. 2002; Miki et al. 1998a,b, 2000; Noguchi et al. 1995; Persson et al. 1993; Sun et al. 2001). The population of nociceptive neurons in the dorsal column was increased after sciatic nerve ligation (Miki et al. 2000). Chronic constriction injury (CCI) of the sciatic nerve induces changes in molecular events in dorsal-column neurons (Miki et al. 1998a; Noguchi et al. 1995). The myelinated fibers begin to produce SP and calcitonin gene–related peptide (CGRP) immunoproducts. In the peripheral nervous system of the aged humans and animals, various pathological changes and a decrease in number of myelinated fibers are reported. Fujisawa and Shiraki (1978) also reported dystrophic changes in the gracile nucleus (GN) of the aged rat. The morphological changes in the peripheral and CNS observed in the aged rats were thought to be equivalent to peripheral nerve injury such as demyelination. Indeed, it has been reported that myelinated nerves are demyelinated and the conduction velocity of those fibers is prolonged with advancing age (Cavallotti 2003; Fundin et al. 1997; Knox et al. 1989; Phillips et al. 2003; Samorajski 1974; Sugiyama et al. 2002). It is highly possible that the morphological changes in the peripheral nervous system in the aged rats reflect the phenotypic changes in the ability to produce neuropeptides. To support this point of view, SP and CGRP were thought to be the possible neuropeptides involved in modulation of GN neuronal activity in aged rats (Miki et al. 1998a; Noguchi et al. 1995). Here we provide evidence that suggests the development of hyperexcitability and increased SP expression in GN neurons in aged rats.

METHODS

The extracellular recording and SP immunohistochemistry were performed on Fisher 344/DuCrj rats in two age groups: 29 to 34 mo old (aged: 294.0 ± 11.4 g, neuronal recording experiments: $n = 9$; SP immunohistochemistry: $n = 10$) and 7 to 12 mo old (adult: 295.2 ± 33.6 g, neuronal recording experiments: $n = 14$; SP immunohistochemistry: $n = 10$). Rats were raised in a pathogen-free environment and kept under food-restricted conditions (fed every other day). The weight of the rats with food restriction was kept about 55% of the freely fed rats and life span was extended for about 25%. The rats were housed three per cage and maintained on a 12:12 light–dark schedule (lights on at 6:00 AM) at 22 ± 2°C (humidity: 55 ± 5%).

The study was approved by the Animal Experimentation Committee at Nihon University School of Dentistry and by the Tokyo Metropolitan Institute of Gerontology. The animals were treated strictly according to the guidelines of the International Association for the Study of Pain (Zimmermann 1983).

Single-neuron recording

ANIMAL PREPARATION. Animals were anesthetized with sodium pentobarbital [50 mg/kg, administered intraperitoneally (ip)] and the trachea and left external jugular veins were cannulated to allow artificial respiration and intravenous administration of drugs, respectively. Anesthesia was maintained with halothane (2–3%) mixed with air (0.1 L/min) during surgery. The rat was mounted on a stereotaxic frame, the medulla was exposed, and a mineral oil pool was made with the skin flaps surrounding the laminectomy. The bipolar concentric electrode was placed in the ventral posterior lateral nucleus of the thalamus (VPL) (Bregma coordinates: P 2.0–3.0 mm; L 2.5–3.5 mm) and the bipolar silver wire electrodes were placed in the sciatic nerve. To position the bipolar electrodes in the VPL, multiple-unit activity was recorded. When the greatest neuronal discharges after mechanical stimulation of the hind-paw region were recorded, the electrodes were fixed with dental acrylic cement to the skull. After surgery, anesthesia was maintained throughout the experiment by continuous inhalation of halothane (1%) mixed with oxygen (0.1 L/min). During recording sessions, rats were immobilized with pancuronium bromide (1 mg · kg^{-1} · h^{-1}, administered intravenously) and ventilated artificially. The expired CO_2 concentration was monitored and maintained between 3.0 and 4.0%. Rectal temperature was maintained at 37–38°C by a thermostatically controlled heating pad (ATB-1100, Nihon Kohden, Tokyo, Japan) and the electrocardiogram was monitored. If the heart rate increased after mechanical or thermal stimulation of the receptive fields, the percentage of halothane was increased.

STIMULATION AND RECORDING. Enamel-coated tungsten microelectrodes (impedance = 10–12 MΩ, 1,000 Hz) were advanced into the GN in 2-μm steps. GN neurons were searched for by applying mechanical stimulation (pressure or brush) to the skin of the hip and leg region. When a single neuron was isolated, the responses to mechanical stimulation of the foot were carefully examined and the receptive field was mapped. Graded mechanical, brush, and pinch stimuli were then applied to the most sensitive areas of the receptive fields with von Frey filaments, camel brush, and small arterial clip, respectively. Tips of the von Frey filaments were modified as illustrated in Fig. 1, to eliminate the response irregularities induced by the

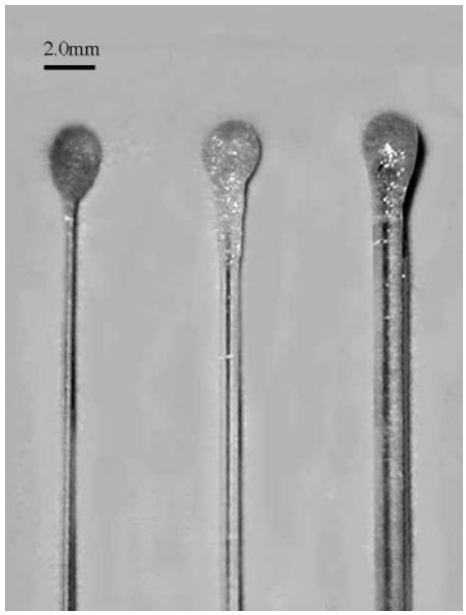

FIG. 1. Photograph of tips of the modified von Frey filaments. Each filament was used for the mechanical stimulation with different stimulus intensity: left filament for 1.2 g, middle filament for 15.1 g, and right filament for 75.9 g.

different size of the contact tips of filaments. Each neuron was classified either as *1*) a wide-dynamic-response (WDR) neuron that responded to both nonnoxious and noxious stimuli and increased its firing frequency as stimulus intensity increased, or as *2*) a nociceptive-specific (NS) neuron that responded exclusively to noxious mechanical stimulation of the receptive fields. After characterization with mechanical stimuli, the responses to thermal stimuli were further examined by heating the most sensitive area of the mechanical receptive field. Before application of the thermal stimulus, the surface temperature was adapted to 38°C for 180 s. Skin heating ranged from 44 to 50°C and lasted 10 s. The rate of temperature change was set at 10°C/s. The thermal stimuli were applied every 190 s to avoid sensitization of the peripheral nociceptors (Beitel and Dubner 1976). GN neurons were tested for antidromic activation after VPL stimulation (pulse duration: 0.2 ms, <0.5 mA). The tip of the thermal probe was 10 mm in diameter. Neuronal activity data were stored on a computer disk for off-line analysis. The sciatic nerve was stimulated to activate A-fibers (pulse duration: 200 μs; intensity: <200 μA) or C-fibers (pulse duration: 2 ms; intensity: 2 mA) and first spike latency was calculated. Antidromic latencies after VPL stimulation were also measured using the collision test. After evaluating the response properties of GN neurons, lesions were made at the recording site by passing negative DC of 10 μA for 10 s for histological identification of the recording site. To avoid sensitization of the receptive field we tested one or two nociceptive neurons with different receptive fields in each rat.

HISTOLOGICAL CONFIRMATION OF THE RECORDING SITE. At the conclusion of the experiment, the rats were overdosed with sodium pentobarbital (100 mg/kg) and perfused transcardially with 50 ml 0.01 M PBS (pH 7.4) followed by 10% formalin in 0.1 M phosphate buffer. The brain was removed, placed in cold fixative for a few days, and then transferred to cold phosphate-buffered 30% sucrose for 48 h. Serial sections (50 μm thick) were cut along the path of the electrode penetration. The sections were counterstained with thionin for identification of the recording and stimulation sites. Camera lucida tracings of the recording and stimulation sites were drawn at 400 × magnification with a drawing tube.

DATA ANALYSIS. The waveforms of single or multiple neuronal activities were analyzed off-line. The waveform of each neuron was identified using Spike2 microcomputer software (CED, Cambridge, UK). Peristimulus time histograms (bin width = 1 s) were generated in response to each stimulus. Background discharges were first recorded for 10 s before application of the mechanical or thermal stimulus and they were subtracted from the neuronal responses during analysis. The spike-density histograms were developed from the interstimulus intervals of background activity for 10 s in GN neurons with high-frequency background activity (>5 Hz) according to the criteria from Kaneoke and Vitek (1996). The burst discharge and irregular firing neurons were defined from the spike-density histograms. Stimulus–response (S-R) functions of each nociceptive neuron were obtained in response to the mechanical (1.2, 5.5, 15.5, 28.8, 75.9 g, pinch and brush) or heat (44–50°C) stimuli. The mechanical or thermal stimulation of the receptive fields was considered to have induced an effect when the peak firing frequency at 5 s after mechanical and 10 s (one trial for each neuron with 180-s intervals) after thermal stimulation differed from the mean background discharge rate by ±2 SD mean firing frequency for 10 s after pinching of the receptive fields was measured and was defined as the afterdischarge. The receptive fields of all neurons were drawn to scale on standard diagrams of a rat leg. Areas of the receptive fields were calculated using image analysis software (National Institutes of Health image 1.61).

SP immunohistochemistry

Aged and adult rats were deeply anesthetized with sodium pentobarbital (80 mg/kg, ip) and perfused through the aorta with 500 ml 0.02 M phosphate-buffered saline (PBS, pH 7.4) followed by 500 ml 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). Medulla and L4–5 dorsal root ganglia (DRG) of the naïve rats were used for the SP immunohistochemistry. The medulla and L4–5 DRG were removed and postfixed in the same fixative for 3 days at 4°C. The tissues were then transferred to 30% sucrose (wt/vol) in PBS for several days for cryoprotection. Sections of the medulla (30 μ thick) were cut with a freezing microtome and every fourth section was collected in PBS. Free-floating tissue sections were rinsed in PBS, 10% normal goat serum in PBS for 1 h, then incubated in rabbit anti-SP (1:10,000; Incstar, Stillwater, MN) for 72 h at 4°C. Next, the sections were incubated in biotinylated goat anti-rabbit IgG (1:200; Vector Labs, Burlingame, CA) for 1 h at 37°C. After washing, the sections were incubated in peroxidase-conjugated avidin–biotin complex (1:100; ABC, Vector Labs) for 4 h at 37°C. After being washed in 0.05 M Tris buffer (TB), the sections were incubated in 0.035% 3,3′-diaminobenzidine-tetra HCl (DAB, Sigma), 0.2% nickel ammonium sulfate, and 0.05% peroxide in 0.05 M TB (pH 7.4). The sections were then washed in PBS, after which the sections were serially mounted on gelatin-coated slides, dehydrated in alcohols, and coverslipped. Rabbit anti-SP (1:500; Peninsula Labs, Belmont, CA) for L4–5 DRG were used as the primary antibody and the staining processes were the same as those for the medulla. For measuring areas occupied by the SP-LI fibers, three sections with dense labeling were randomly chosen from each rat. The areas (100 × 200 μm^2) in the middle portion of the GN were processed using a microcomputer system (National Institutes of Health image 1.61). The mean areas occupied by the SP immunoproducts from these three sections were calculated for each animal. In addition, the mean percentage areas occupied by the SP immunoproducts were calculated by the formula: mean percent area (%) = (area occupied by the SP immunoproducts in 100 × 200 μm^2)/(100 × 200 μm^2) × 100. The area of each DRG cell was calculated by the formula: area = major axis × minor axis × π (3.14) × 1/4.

Statistical analysis

Statistical analysis was performed using ANOVA followed by Newman–Keuls test. Mann–Whitney *U* test or Welch's *t*-test was also used as appropriate. Differences were considered significant at $P < 0.05$. Results are presented as means ± SE.

RESULTS

Spatial distribution of nociceptive neurons in the gracile nucleus

A total of 122 gracile nucleus (GN) neurons were recorded in aged and adult rats (32 LTM, 34 WDR, and two NS neurons in the aged rats; 32 LTM and 22 WDR neurons in the adult rats). Because brush and gentle pressure were used as search stimuli, there might be bias toward finding WDR units. In fact, only two NS neurons were recorded from the aged rats. Thus we focused on analyzing the physiological properties of WDR neurons. A dorsal view of the medulla is shown in Fig. 2, *A* (aged) and *B* (adult). The penetration tracks where nociceptive neurons were encountered are plotted on the surface of the medulla. Nociceptive neurons were rostrocaudally distributed in the GN as illustrated in Fig. 2, *C* and *D*. The even distribution of the recorded nociceptive neurons in aged and adult rats was similar.

Antidromic responses from ventral posterior lateral nucleus of thalamus

Figure 3 illustrates an example of the antidromic responses of WDR neurons recorded from the GN of 32- and 9-mo-old

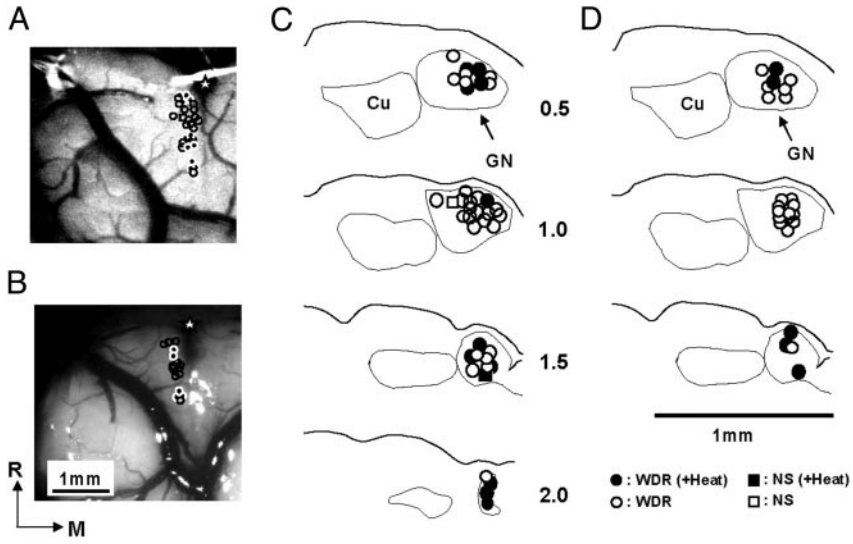

FIG. 2. Distribution of nociceptive neurons in the gracile nucleus (GN). Photograph of a dorsal view of the medulla of 32-mo-old rat (*A*) and 9-mo-old rat (*B*). Horizontal sections of the medulla and location of nociceptive neurons in the GN of aged rats (*C*) and adult rats (*D*). Numbers on the *left column* in *B* indicate the distance from the obex. Stars in *A* and *C* indicate the location of the obex. Cu, cuniate nucleus; WDR, wide-dynamic-range neurons; NS, nociceptive-specific neurons.

(Fig. 3, *A* and *B*, respectively) rats and the stimulation sites in the ventral posterior lateral nucleus of thalamus (VPL) are illustrated in Fig. 3, *D* and *E*. The response latencies to antidromic activation were analyzed when stimulation sites were located within the VPL. The thalamic projection was identified by the following criteria: *1*) the response latencies to VPL stimulation were consistent, *2*) the neuron was able to follow ≥333-Hz stimuli (Fig. 3, *Aa* and *Ab*), and *3*) the response collides with the orthodromic responses after sciatic nerve stimulation (Fig. 3, *Ac–e*). Mean antidromic response latencies were similar in aged and adult rats (aged: 2.4 ± 0.4 ms, $n = 8$; adult: 1.8 ± 0.2 ms, $n = 4$) (Fig. 3*C*, $P > 0.05$, Welch's *t*-test). Thus there is no change in conduction velocity of the thalamic projecting axons in aged rats.

The orthodromic response latencies after sciatic nerve stimulation

We obtained two different orthodromic responses of short (<20 ms; Fig. 4*A*) and long (>20 ms; Fig. 4*C*) latencies after electrical stimulation of the sciatic nerve. The short-latency responses (aged: 9.3 ± 0.6 ms, $n = 29$; adult: 8.9 ± 1.3 ms, $n = 16$) were not different between the aged (Fig. 4, *Aa* and *B*) and adult rats (Fig. 4, *Ab* and *B*) ($P > 0.05$, Welch's *t*-test). In contrast, the long-latency response of the aged rats (116.3 ± 9.2 ms, $n = 18$) (Fig. 4, *Ca* and *D*) was significantly longer than that of the adult rats (89.4 ± 8.5 ms, $n = 8$, $P < 0.05$, Welch's *t*-test) (Fig. 4, *Cb* and *D*).

Background activity

Background firing of the aged and adult rats showed different patterns as illustrated in Fig. 5, *A* and *B*. Based on the spike density analysis, we classified GN WDR neurons with high-frequency background activity (>5 Hz, aged: 20/34, adult: 6/22) as burst discharge neurons. The proportion of GN WDR neurons exhibiting a burst discharge pattern is higher in aged rats (14/20) than that in adult rats (2/6). Many WDR neurons showed high-frequency background activity in aged rats (Fig. 5*C*). The mean background activity (aged: 12.4 ± 2.4 spikes/s,

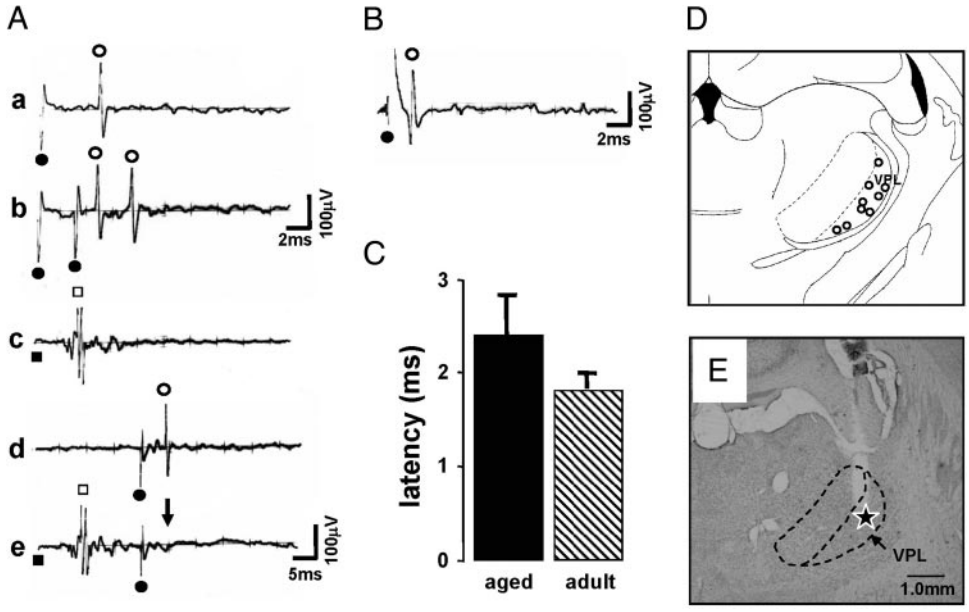

FIG. 3. Antidromic responses of GN WDR neurons after ventral posterior lateral (VPL) stimulation in aged (*Aa*) and adult (*B*) rats. Spikes of this neuron were followed by 333-Hz electrical shocks (*Ab*). *Ac*: orthodromic spikes. *Ad*: antidromic spike after VPL stimulation. Note differences in the stimulus onset and sweep time in *Aa* and *Ad*. *Ae*: antidromic spike was abolished after the orthodromic spikes. Arrow in *Ae* indicates the expected time point for the antidromic spike. *C*: mean antidromic latencies of aged and adult rats ($P > 0.05$, Welch's *t*-test). Solid circles and squares indicate the onset of the VPL and sciatic nerve stimulation, respectively. Open circles and squares indicate the antidromic and orthodromic spikes. *D*: camera lucida drawings of stimulation sites in VPL. *E*: photomicrograph of the electrode tip in VPL. Star indicates the electrode tip in VPL. VPL, ventral posterior lateral nucleus of thalamus.

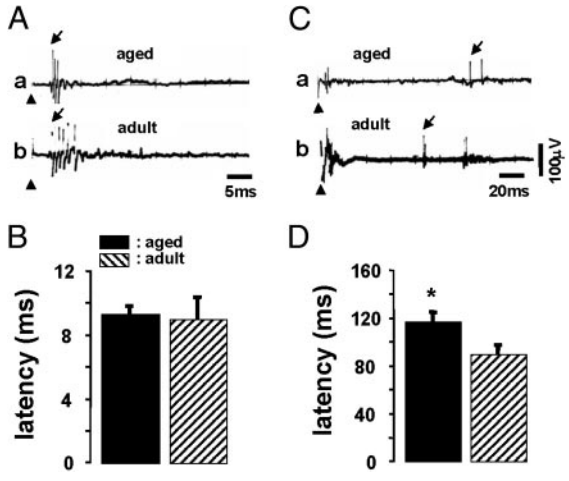

FIG. 4. A-fiber (*A*) and C-fiber (*C*) orthodromic responses of GN WDR neurons after sciatic nerve stimulation. *B*: latencies of A-fiber responses were not different between aged and adult rats ($P > 0.05$, Welch's *t*-test). *D*: significant difference between aged and adult rats was shown in latencies of C-fibers (*$P < 0.05$, Welch's *t*-test). Arrowheads indicate the stimulus onset and the arrows indicate the first spikes elicited by sciatic nerve stimulation.

$n = 34$; adult: 4.2 ± 1.5 spikes/s, $n = 22$, $P < 0.01$) was significantly higher in aged rats than that in adult rats ($P < 0.01$, Welch's *t*-test, Fig. 5*D*).

Mechanical and thermal responses

We did not observe any differences in responses to brushing of the receptive fields in aged and adult rats (Fig. 6, *A* and *B*). GN nociceptive neurons increased firing frequency after graded mechanical stimulation of the hind paw both in aged and adult rats (Fig. 6, *A* and *B*). The stimulus–response curve of the mechanical responses was significantly different in aged and adult rats (Fig. 6*B*) ($P < 0.05$, Newman–Keuls test). Furthermore, the response magnitude to mechanical stimulation in aged rats was significantly greater than that of adult rats when the stimulus intensity was >79.9 g or pinching was applied. In both aged and adult rats, GN nociceptive neurons gradually increased firing frequency after increases in stimulus temperature. The firing pattern of GN neurons during skin heating was similar in aged and adult rats (Fig. 6*C*), although the heat-evoked responses were significantly greater in aged rats than in adult rats ($P < 0.05$, Newman–Keuls test, Fig. 6*D*).

Figure 7*A* shows histograms of the afterdischarge following pinching of the receptive fields in aged (Fig. 7*Aa*) and adult rats (Fig. 7*Ab*). We observed high-frequency background activity after pinching of the receptive field in the aged rats. The afterdischarges that lasted >100 s were occasionally seen in aged rats. The afterdischarge (aged: 14.1 ± 2.5 spikes/s, $n = 34$; adult: 6.1 ± 1.6 spikes/s, $n = 22$, $P < 0.05$) was signifi-

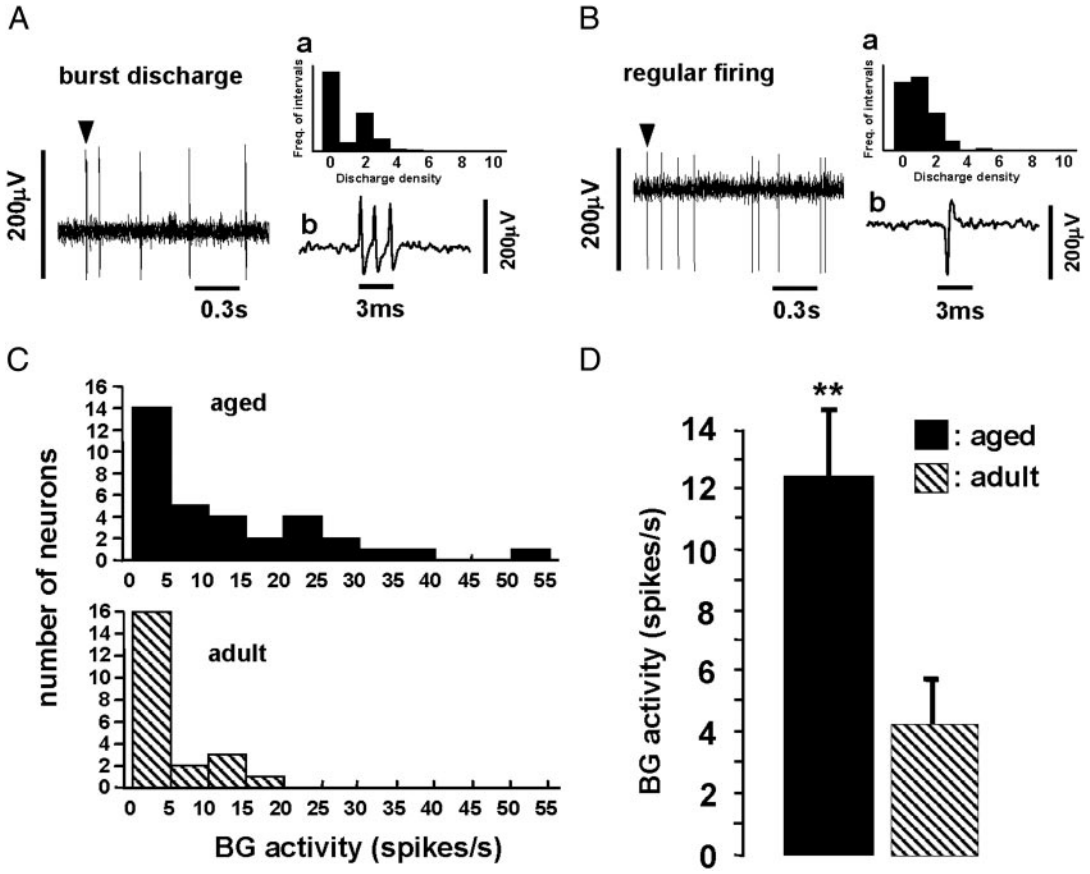

FIG. 5. Background activity of GN WDR neurons in the aged and adult rats. Typical examples of background activities of burst discharge (*A*) and regular firing GN WDR neurons (*B*) recorded from the aged rats. Expanded sweep time tracing of firings are indicated in *Ab* and *Bb*. *Inset histograms* (*A* and *B*): typical examples of spike density histograms of burst discharge (*Aa*) and regular firing GN neurons (*Ba*) in aged rats. *C*: frequency histograms of firing frequencies of background activity (aged: *top trace*; adult: *bottom trace*). *D*: mean background activity was significantly higher in aged rats than that in adult rats (**$P < 0.01$, Welch's *t*-test). BG activity, background activity. Arrowheads indicate the spikes expanded in *A* and *B*.

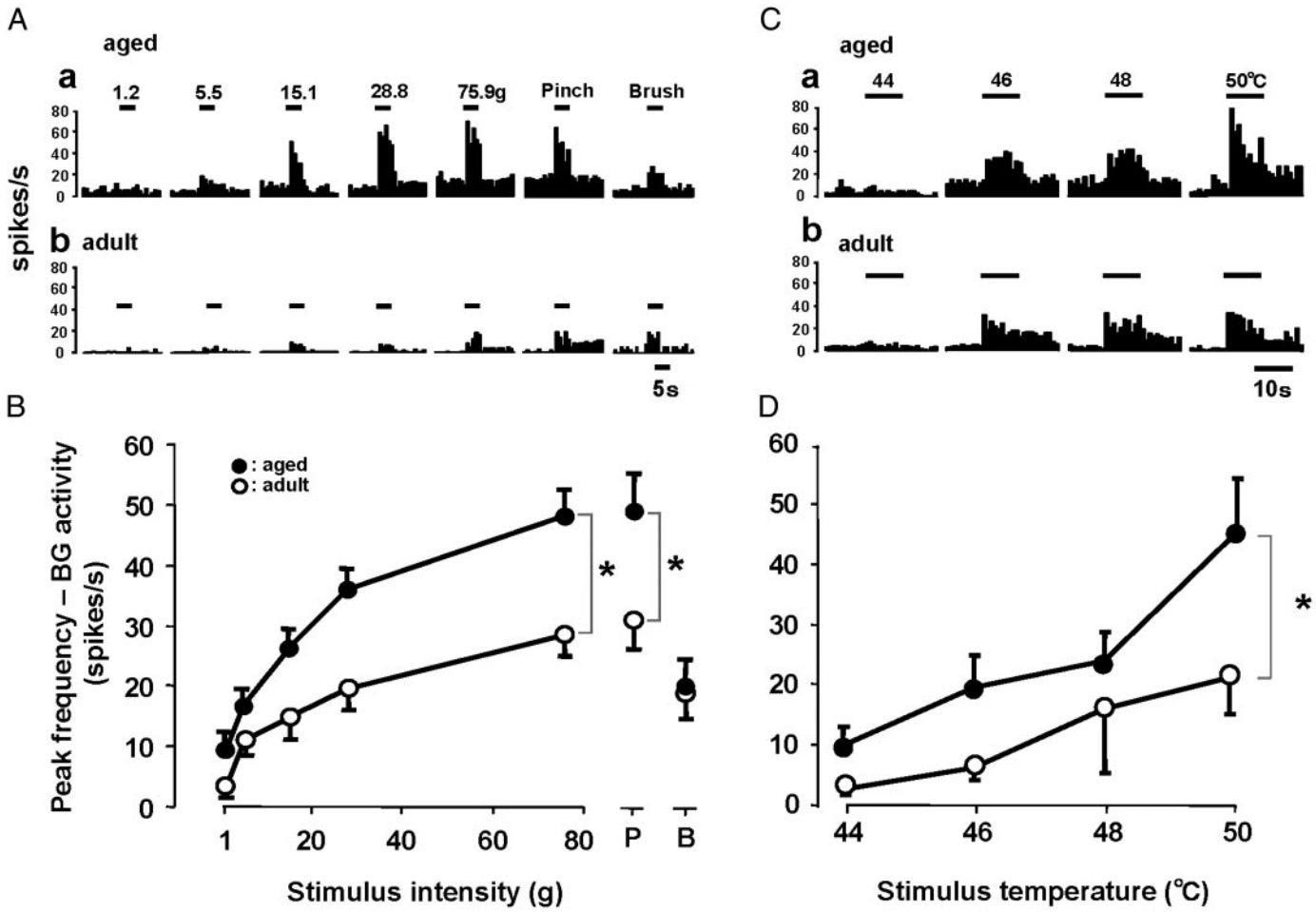

FIG. 6. *A* and *B*: stimulus–response function of responses of GN WDR neurons to graded mechanical stimulation in the aged and adult rats. Mechanical responses were significantly larger in aged rats (*Aa* and solid circles in *B*) than in adult rats (*Ab* and open circles in *B*) (*$P < 0.05$, Newman–Keuls test). *C* and *D*: stimulus–response function of responses to graded heat stimulation in the aged and adult rats. Heat responses were significantly larger in aged rats (*Ca* and solid circles in *D*) than in adult rats (*Cb* and open circles in *D*) (*$P < 0.05$, Newman–Keuls test). P, pinch; B, brush.

cantly higher in aged rats than that in adult rats ($P < 0.05$, Welch's *t*-test, Fig. 7*B*).

Receptive field properties

Based on responses to mechanical stimulation, it has been reported that the center of the receptive fields of WDR neurons is a low-threshold zone and the peripheral region is a high-threshold region (Kondo et al. 2002; Miki et al. 1998b). We analyzed the receptive field profile based on the size of the low- and high-threshold areas. The receptive fields of GN nociceptive neurons were located on the ventral and dorsal surfaces of the hind paw in aged and adult rats. There were no significant differences in the size of the receptive fields of low- and high-threshold areas between the aged and adult rats (Table 1).

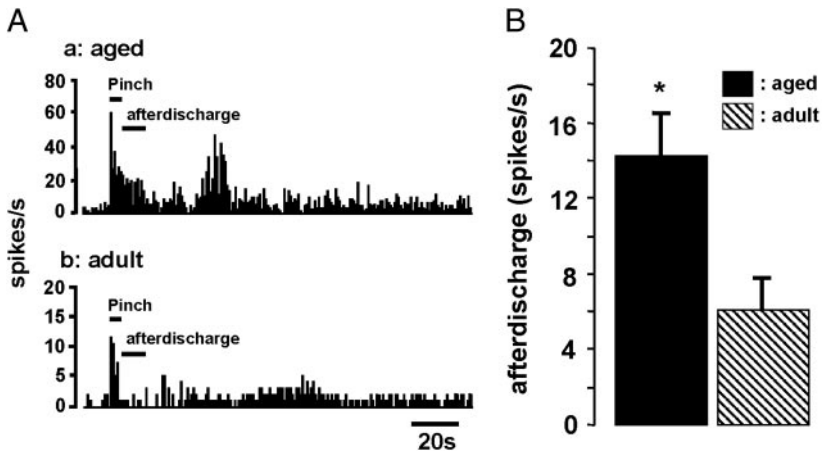

FIG. 7. Afterdischarge of GN WDR neurons in aged and adult rats. *A*: typical examples of poststimulus time histograms of WDR neurons after pinching of the receptive fields (a, aged; b, adult). Mean afterdischarge of GN WDR neurons is illustrated in *B*. Afterdischarges are significantly higher in aged rats than in adult rats (*$P < 0.05$, Welch's *t*-test).

TABLE 1. *Mean areas of the receptive fields of GN WDR neurons in aged and adult rats*

		Threshold Area, cm^2	
	n	Low	High
Aged	34	0.8 ± 0.6	2.2 ± 1.0
Adult	22	0.6 ± 0.5	1.5 ± 0.9

Values are ± SE. GN, gracile nucleus; WDR, wide-dynamic range.

SP-like immunoreactivity

We observed dense labeling of SP-LI terminals in the GN of aged rats (Fig. 8*A*). SP-LI processes were distributed in the GN extensively in the aged rats (Fig. 8*A*); however, sparse labeling of SP-LI fibers was observed in the adult rats. The density of SP-LI fibers was significantly higher in aged rats than in adult rats, as illustrated in Fig. 8*B* (aged: 14.1 ± 2.1, $n = 5$; adult: 4.2 ± 1.6, $n = 5$, $P < 0.05$, Mann–Whitney *U* test). The photomicrographs and mean areas of SP-LI DRG neurons are illustrated in Fig. 9. SP-LI DRG neurons were significantly larger in aged rats than in adults (aged: 804.2 ± 14.2 μm^2, $n =$ 262 from five rats; adult: 605.0 ± 15.1 μm^2, $n = 159$ from five rats, $P < 0.01$, Welch's *t*-test).

DISCUSSION

The spinothalamic system is an important CNS pathway for pain transmission (Chung et al. 1979; Kenshalo et al. 1979, 1982; Willis and Coggeshall 1991), whereas GN neurons have an important function in conveying innocuous cutaneous, proprioceptive, and visceral sensory information to the higher CNS (Cliffer et al. 1992; Ferrington et al. 1988; Giesler and Cliffer 1985). Most GN neurons responded to gentle mechanical stimulation of the foot skin. Only a small number of neurons in the GN responded to noxious stimulation. However, recent studies show that GN neurons exhibit increased background activity and afterdischarges following sciatic nerve injury (Kondo et al. 2002; Miki et al. 1998b). The incidence of WDR neurons is increased in the GN of the rats with sciatic nerve ligation. Furthermore, heat responses are frequently recorded from GN neurons in rats with sciatic nerve ligation (Kondo et al. 2002; Miki et al. 1998b). Allodynia-like behavior in rats with sciatic nerve ligation is abolished after the ablation of the GN or lidocaine injection into GN (Sun et al. 2001). It has been reported that myelinated nerves are demyelinated and the conduction velocity of these fibers is prolonged in aged rats (Cavallotti 2003; Fundin et al. 1997; Knox et al. 1989; Phillips et al. 2003; Samorajski 1974; Sugiyama et al. 2002). The morphological changes in the primary afferent fibers should reflect the excitability of these fibers. It is possible that the changes in physiological property of primary afferent fibers will affect the GN neuronal activity with advancing age.

Response property of GN neurons

It has been reported that GN neurons receive cutaneous input through DC and postsynaptic dorsal column (PSDC) pathways. Wall and Dubner (1972) also reported that the DC system would be involved in multiple somatosensory functions such as vibration sensitivity, weight discrimination, touch sensitivity, two-point discrimination, roughness discrimination, and stereognosis. These multiple functions of the DC system were thought to be involved in an innocuous somatosensory discrimination. Furthermore, a part of the DC pathway is believed to be mainly involved in cutaneous nociception. On the other hand, the PSDC pathway is known to be dominantly involved in viscera and some in cutaneous input (Al-Chaer et al. 1997; Willis et al. 1999). Our results showed an increase in the activity of GN neurons in aged rats that received input from the foot skin. It is thus likely that an increase in GN neuronal activity in aged rats may be induced by the input through the DC pathways. However, we cannot rule out a possibility that an effect of aging on visceral nociception induces an increase in GN neuronal activity in aged rats (Al-Chaer et al. 1996).

We observed the dense labeling of SP immunoreactive fibers in the GN and the large-diameter DRG neurons labeled with SP antibody in the aged rats, which is in sharp contrast with that in adult rats. It is likely that these differences may result from an increased excitability of GN nociceptive neurons. In addition, the loss of serotonin and dopamine β-hydroxylase (DBH) immunoreactive fibers may also be involved in the increase in the excitability of DH nociceptive neurons (Iwata et al. 1995, 2002). The background activity, afterdischarge and heat-evoked responses have very similar properties in aged GN and DH nociceptive neurons, except mechanically evoked re-

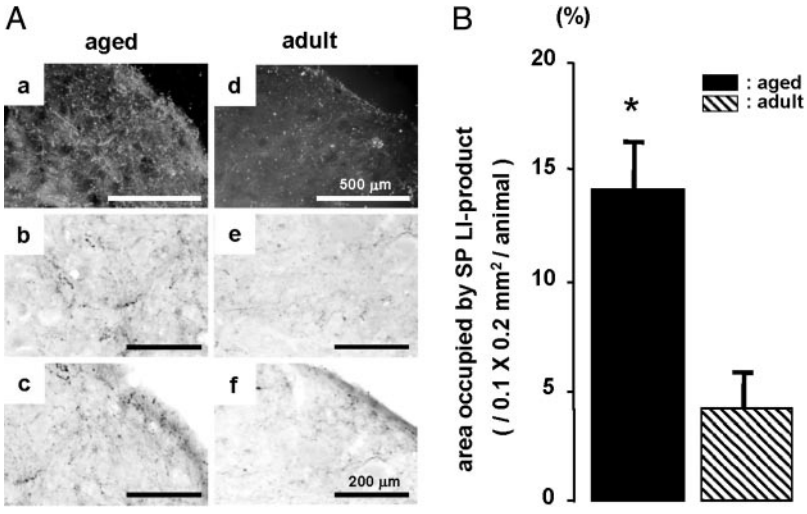

FIG. 8. Substance P–like immunoreactivity (SP-LI) fibers in the GN of the aged and adult rats. *A*: photomicrographs of the GN in aged and adult rats. Dense labeling of SP-LI terminals was observed in the GN of the aged rats, whereas sparse labeling was observed in the adult rats: a (aged) and d (adult): dark field photomicrographs; b (aged) and e (adult): light-field photomicrographs of the center of GN; c (aged) and f (adult): light-field photomicrographs of the marginal area of GN. *B*: mean area occupied by the SP immunoproducts. Area occupied by the SP immunoproducts was significantly larger in the aged rats than that in the adult rats (*$P < 0.05$, Mann–Whitney *U* test).

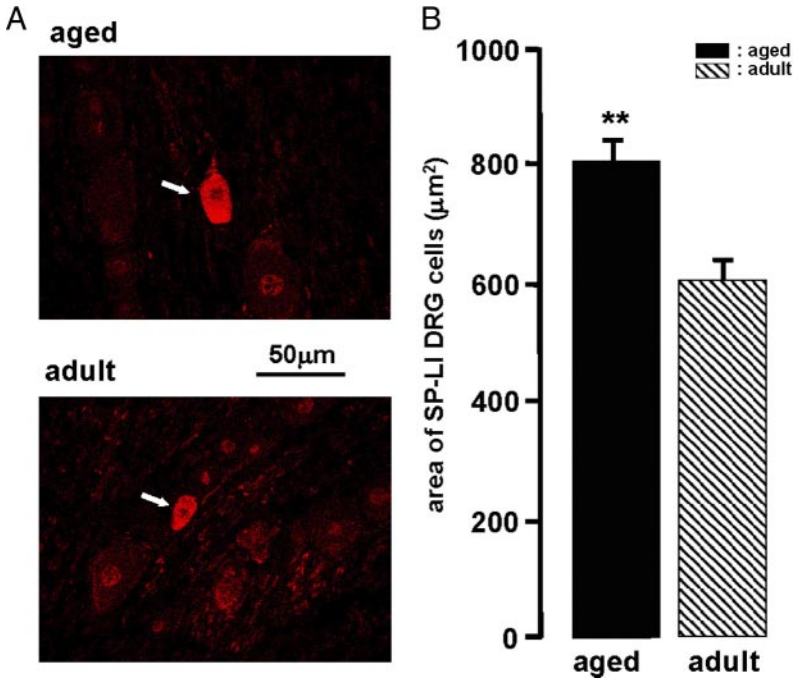

FIG. 9. SP-LI L4–5 dorsal root ganglia (DRG) cells of the aged and adult rats. *A*: photomicrographs of the L5 DRG cells in aged and adult rats. Arrows indicate the typical SP-LI DRG cells. *B*: area of SP-LI DRG cells. SP-LI cells were significantly larger in aged rats than those in adults (**$P < 0.01$, Welch's *t*-test).

sponses and the receptive field size. The different underlying mechanisms may be involved in the differential effects of aging on heat and mechanical responses in DH and GN nociceptive neurons of the aged rats.

Morphological change in GN neurons

Noguchi et al. (1995) and Miki et al. (1998a) reported that sciatic nerve transection induced SP or CGRP-LI in large dorsal root ganglion neurons. They suggested that peripheral nerve injury induced SP and CGRP immunoproducts in myelinated large-diameter nerve fibers. Similarly, in the present experiments, we also found dense labeling of SP-LI axons in the GN of the aged rats, whereas sparse labeling was observed in the adult rats. Furthermore, the SP-LI DRG neurons were significantly larger in the aged rats. These results suggest that SP-LI primary afferents would be involved in an extensive increase in the excitability of GN neurons in aged rats. A large number of Fos protein–LI cells were observed in the GN of the aged rats after peripheral inflammation. This Fos protein induction in GN neurons after inflammation is thought to be a result of SP release from primary afferent terminals in the aged rats. Thus the morphological changes observed in the GN of the aged rats may be produced by peripheral nerve injury.

Physiological and morphological changes with advancing age have been reported in peripheral nerves (Cavallotti 2003; Fundin et al. 1997; Knox et al. 1989; Ochoa and Mair 1969; Phillips et al. 2003; Samorajski 1974; Sugiyama et al. 2002). A decrease in the number of large-diameter myelinated fibers, abnormal myelin sheath, and axon collapse was found in aged peripheral nerves (Cavallotti 2003; Fundin et al. 1997; Knox et al. 1989; Phillips et al. 2003; Samorajski 1974; Sugiyama et al. 2002). The response latency is also prolonged in the aged rats. Therefore it is very likely that the functional and morphological changes in primary afferent myelinated nerve fibers would affect the excitability of GN neurons in the aged rats, shown as an increase in GN neuronal activity.

It has recently been reported that γ-aminobutyric acid (GABA)–receptor-mediated current is inhibited by substance P pretreatment in the dissociated DRG neurons (Si et al. 2004). Furthermore, the GN neuronal activity was enhanced after topical application of picrotoxin in the GN, suggesting that GABAergic system would be involved in GN neuronal excitability in correlation with the SP system (Berkley and Hubscher 1995). These findings suggest that the GABAergic system is involved in the modulation of SP immunoreactivity in the peripheral and CNS. It is probable that the increase in SP-LI fibers in the GN and the appearance of large-diameter SP-LI DRG neurons in aged rats may be modulated by the GABAergic system.

In summary, we have demonstrated significant differences in the GN of aged and adult rats as follows. *1*) The long-latency responses elicited by the sciatic nerve stimulation showed longer latency in aged rats than in adults. *2*) The background activity and afterdischarges were substantially higher in aged rats. *3*) The heat response was significantly larger in aged rats than in adults. *4*) The nonnoxious brush, pressure responses, and mechanical threshold were not different in aged and adult rats. On the other hand, the noxious mechanical response was significantly greater in aged rats than in adults. *5*) A large number of SP-LI fibers were distributed in the GN of the aged rats. *6*) SP-LI DRG neurons were significantly larger in aged rats than in adult rats. On the other hand, antidromic latency to VPL stimulation, short-latency orthodromic responses to sciatic nerve stimulation, and receptive field size were not different between the aged and adult rats.

Our results suggest that the increase in noxious mechanical and heat responses of GN nociceptive neurons would arise from underlying mechanisms of mechanical and thermal hyperalgesia in elderly people.

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