

Age-related physiological and morphological changes of muscle spindles in rats

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Age-related physiological and morphological changes of muscle spindles were examined in rats (male Fischer 344/DuCrj; young, 4–13 months; middle-aged, 20–22 months; old, 28–31 months). Single afferent discharges of the muscle spindles in gastrocnemius muscles were recorded from a finely split dorsal root during ramp-and-hold (amplitude, 2.0 mm; velocity, 2–20 mm s⁻¹) or sinusoidal stretch (amplitude, 0.05–1.0 mm; frequency, 0.5–2 Hz). Respective conduction velocities (CVs) were then measured. After electrophysiological experimentation, the muscles were dissected. The silver-impregnated muscle spindles were teased and then analysed using a light microscope. The CV and dynamic response to ramp-and-hold stretch of many endings were widely overlapped in old rats because of the decreased CV and dynamic response of primary endings. Many units in old rats showed slowing of discharge during the release phase under ramp-and-hold stretch and continuous discharge under sinusoidal stretch, similarly to secondary endings in young and middle-aged rats. Morphological studies revealed that primary endings of aged rat muscle spindles were less spiral or non-spiral in appearance, but secondary endings appeared unchanged. These results suggest first that primary muscle spindles in old rats are indistinguishable from secondary endings when determined solely by previously used physiological criteria. Secondly, these physiological results reflect drastic age-related morphological changes in spindle primary endings.

(Resubmitted 8 February 2007; accepted after revision 3 May 2007; first published online 10 May 2007)

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Postural sway and gait stability deterioration that occur with ageing have been studied extensively (Overstall *et al.* 1977; Alexander, 1994; Hausdorff *et al.* 1997; Hurley *et al.* 1998). It has been considered that peripheral proprioceptors, particularly muscle spindles, play an important role in the detection of both passive and active movements (Proske *et al.* 2000), maintenance of postural stability (Lord & Ward, 1994), and control of posture and balance during the swing phase of locomotion (Sorensen *et al.* 2002). For that reason, it is probable that age-related changes of muscle spindles engender a decline in postural and locomotion control among elderly people. A previous physiological study (Miwa *et al.* 1995) revealed that the dynamic and static length sensitivities of muscle spindle primary endings in response to ramp stretch were decreased in aged rats. Furthermore, recent morphological studies have revealed that sensory nerve endings which are immunoreactive to PGP9.5 showed degeneration with ageing (Yamamoto *et al.* 2003; Winarakwong *et al.* 2004). However, little is known about actual effects of ageing on the morphology-function relationship in muscle spindles. Functional and structural changes reportedly

occur in peripheral nerves of old subjects: a decline in nerve conduction velocity (Lafratta & Canestrari, 1966; Dorfman & Bosley, 1979; Chase *et al.* 1992), axonal atrophy (Ochoa & Mair, 1969; Knox *et al.* 1989; Chase *et al.* 1992), a decline in internodal length (Lascelles & Thomas, 1966), and demyelination (Knox *et al.* 1989; Adinolfi *et al.* 1991). Age-related function and structural alterations at the neuromuscular junction and in spinal cord motor neurons have also been investigated: skeletal muscle denervation and re-innervation, and motor unit remodelling or loss in ageing rats or humans (Hashizume *et al.* 1988; Kanda & Hashizume, 1989, 1992; Einsiedel & Luff, 1992; Doherty *et al.* 1993). Functional deficits might be the consequence of structural changes that engender a slowly progressive loss of neurons and nerve fibres (Verdu *et al.* 2000). The present study was intended to extend the knowledge of age-related changes in physiological properties and structure in muscle spindles.

Muscle spindles have two kinds of endings, which are supplied, respectively, by Ia and II fibres: primary and secondary endings. Morphologically, primary endings consist of spiral or annular terminations on each

intrafusal fibre in the equatorial region, whereas secondary endings have extensive irregular spiral or annular terminations on chain fibres and some spray-like terminations on bag fibres in the juxta-equatorial region (Ruffini, 1898; Boyd, 1962, Boyd, 1985; Banks *et al.* 1982; Hunt, 1990). Electrophysiological experiments have classified primary and secondary endings, usually based on their differences in conduction velocity (CV) and response to ramp-and-hold or sinusoidal stretches. Primary endings show faster CV, higher dynamic response, and a narrower linear range on sinusoidal stretch. In contrast, secondary endings show slower CV, lower dynamic response, and a broader linear range on sinusoidal stretch (Matthews, 1963; Matthews & Stein, 1969; Hunt & Ottoson, 1975; Cheney & Preston, 1976; Barker *et al.* 1986; Wei *et al.* 1986; Scott, 1990; De-Doncker *et al.* 2003). Miwa *et al.* (1995) assumed that muscle spindles showing abrupt cessation of firing during the release phase were primary endings and that those showing slowing of firing were secondary endings. However, that study examined few samples. Moreover, different from the case of young rats, it was difficult to use the CV and dynamic index in old rats because no clear transition point was found. The possibility remains that the sample of Miwa and colleagues was biased in preference to select in favour of secondary endings. Whether or not the current criteria are generally available for classification of primary and secondary endings in muscle spindles of old rats remains to be clarified. The first aim of the present study is to examine whether or not electrophysiological criteria, e.g. CV, response to ramp-and-hold and response to sinusoidal stretches, or their combination, are useful for classifying muscle spindles of old rats into primary and secondary endings. The muscle depolarizing drug succinylcholine (SCh) has been used for distinguishing the afferents of primary and secondary endings: SCh greatly increases the dynamic response of primary endings to muscle stretch relative to that of secondary endings (Rack & Westbury, 1966; Cody *et al.* 1972; Inoue *et al.* 1981; Gregory & Proske, 1987; Kishimoto *et al.* 1998). In addition, we attempted to classify spindle endings by their dynamic response change following injection of SCh. The second aim of this study is the investigation of age-related morphological changes in sensory endings by teased silver-impregnated muscle spindles. Through addition of morphological quantitative studies of spindle afferents, we re-examined age-related changes of muscle spindles in old rats.

Methods

Animals

Experiments used male Fischer 344/DuCrj rats of three age groups: young (4–13.5 months of age; body weight, 412.2 ± 41.3 g; 36 rats), middle-aged (20–22 months of

age; body weight, 434.2 ± 21.5 g; 13 rats), and old (28–31 months of age; body weight, 388.9 ± 37.9 g; 19 rats). They had been raised under SPF conditions with *ad libitum* access to food. The mean survival time of this strain in the animal facility is about 28 months. All procedures were approved by the Committee of Animal Care of the Tokyo Metropolitan Institute of Gerontology.

Surgical and experimental procedures

Rats were anaesthetized using pentobarbital sodium (young, middle-aged, 50 mg kg^{-1} i.p.; old, 25 mg kg^{-1} i.p.). Adequate depth of anaesthesia was monitored frequently by checking the pupil size and the flexion reflex to paw pinch. Especially, before giving the muscle relaxant (SCh), it was confirmed that anaesthesia was sufficiently deep to suppress the flexion reflex to paw pinch. Supplementary injections (10 mg kg^{-1}) were administered when necessary. After the experiment, each rat was killed using an overdose of pentobarbital sodium (100 mg kg^{-1}).

The trachea, a common carotid artery and an external jugular vein were cannulated. The rats were ventilated artificially and their arterial blood pressure was monitored. The left gastrocnemius muscle was isolated from the surrounding tissue. Except for the nerve to the gastrocnemius muscle, the left hip and hindlimb muscles were denervated. The calcaneal tendon was removed from the calcaneum and was attached to a stretcher using a suture hook. Before detaching the tendon from the calcaneum, the gastrocnemius muscle length was measured. The leg and lumbosacral spine were immobilized in a metal frame using pins and clamps. The lumbosacral spinal cord was exposed by a laminectomy and both ventral and dorsal roots L4–S1 were severed near the entry to the spinal cord. The exposed area of the spinal cord and limb were covered with pools of mineral oil warmed to $35\text{--}38^\circ\text{C}$. Rectal temperature was monitored and controlled to $36\text{--}38^\circ\text{C}$ using a heating blanket and infrared radiation. In some experiments, lactated Ringer solution (Otsuka Pharmaceutical Co. Ltd) or 4% Ficoll (Pharmacia Fine Chemicals) solution was infused ($0.69\text{--}1.3 \text{ ml h}^{-1}$) to maintain adequate blood pressure. Single afferent discharges of the muscle spindles in the gastrocnemius muscle were recorded from a finely split dorsal root (L4–L6). Muscle spindles were distinguished from Golgi tendon organs by their different discharge behaviour during a twitch. The Golgi tendon organs are situated in series with the extrafusal muscle fibres and were thus excited by the twitch. The muscle spindles, however, lie in parallel with the extrafusal muscle fibres and were therefore paused by the twitch.

The initial muscle length was set at a length that generates muscle tension of about 50 g. The muscle was stretched by 2.0 mm at 2, 4, 10 and 20 mm s^{-1} , followed by a 5 s plateau and a 1 s release phase. In

addition, the muscle was stretched using a sinusoidal wave (amplitude, 0.02–2.0 mm; frequency, 0.5–2.0 Hz). Each series of stretches was repeated six times every 15 s. In some experiments, the effects of SCh on the stretch responses were tested by i.v. injection of SCh ($200 \mu\text{g kg}^{-1}$) during ramp-and-hold stretches (2 mm at 10 mm s^{-1}). Muscle spindles' afferent spikes were recorded using a digital tape recorder (Instrumentation Cassette Recorder PC-108; Sony Corp., Japan) for storage. The CV of each afferent fibre was calculated from latency, which was determined by backward spike-triggered averaging from the gastrocnemius nerve and the conduction distance, as measured at the end of the experiment. After electrophysiological experimentation, the medial gastrocnemius muscle was dissected and immersed immediately in freshly prepared fixing solution (chloral hydrate, 1 g; 95% alcohol, 45 ml; distilled water, 50 ml; conc. nitric acid, 1 ml). The silver impregnation staining method was used to carry out morphological observation (Barker & Ip, 1963).

Electrophysiological analysis of muscle spindle afferents

The recorded afferent discharges were treated as cluster cuts using Discovery software (DataWave Technologies Corp., Longmont, CO, USA) to classify spike waveforms, if not single units. The instantaneous discharge frequency was calculated as the inverse of the interspike interval. Typical responses of muscle spindle afferents to ramp-and-hold stretch at 2 mm s^{-1} are illustrated in Fig. 1. The peak frequency (PF) was the maximum frequency at the end of the dynamic stretch. The dynamic index (DI) represents differences between the PF and the frequency at 0.5 s after completion of the stretch (Crowe & Matthews, 1964). We classified the recorded muscle spindle afferents into Absence (Fig. 1A) and Presence units (Fig. 1B) according to their responses during the release phase, which showed either absence or presence of the slowing discharge (Hunt & Ottoson, 1975; Hunt, 1990; De-Doncker *et al.* 2003). We performed preliminary tests at different velocities of release as 0.5, 1, 2, 4, 8 and 10 mm s^{-1} to determine the most effective separation of the ending types. Responses of the release phase were apparently dependent on the velocity of release. All showed slowing discharge at slower velocity (0.5, 1 mm s^{-1}), and a paused discharge at faster velocity (4, 8, 10 mm s^{-1}). However, at 2 mm s^{-1} , most afferents of slow conduction velocity units showed slowing discharge, and most afferents of fast conduction velocity units showed an interruption of discharge. Therefore, we used 2 mm s^{-1} as the velocity of release in these experiments. The units were not included in the sample when there were afferents with intermediate properties which were classifiable as neither Presence nor Absence type at this release velocity.

In addition, muscle spindle afferents were characterized using the following criteria: CV, dynamic sensitivity to ramp-and-hold stretches, effect of SCh during ramp-and-hold stretches, and the linear range in response to sinusoidal stretches. Dynamic sensitivity was expressed as the relationship between DI and stretch velocities. We estimated the slope of the regression lines (exponent coefficient) on a double-logarithmic coordinate system. The effect of SCh during ramp-and-hold stretches was estimated by comparing DI before and after i.v. injection of SCh ($200 \mu\text{g kg}^{-1}$). For sinusoidal stretch, the sine curve was fitted to the discharge rate using the least mean square method, as used in previous studies (Matthews & Stein, 1969; Kakuda, 2000), and the mean level and response amplitude (half-peak-to-peak amplitude) were measured (Fig. 2A). We estimated the linear range within which the response amplitude of afferents to the amplitude of stretch

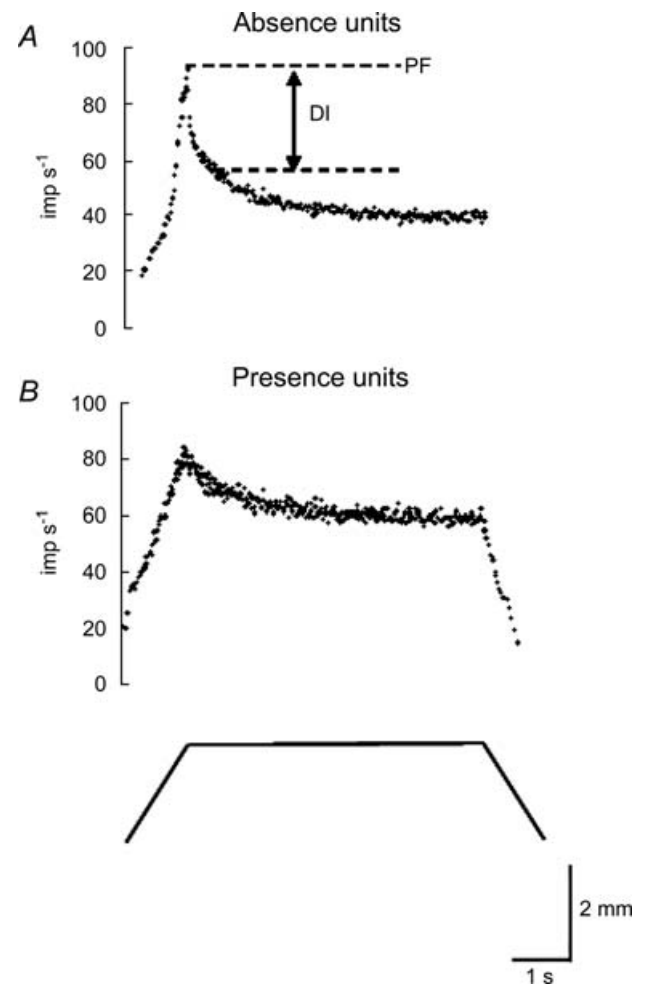


Figure 1. Instantaneous discharge of muscle spindle afferents to ramp-and-hold stretch at 2 mm s^{-1}
 A, discharge ceases during the release phase (Absence units).
 B, discharge continues during the release phase (Presence units). The lower trace shows the muscle displacement.
 PF, peak frequency; DI, dynamic index.

is highly linear. Figure 2B shows that, at a fixed frequency (0.5 Hz), the linear response of Absence units was limited to the low amplitude of stretch, but the linear response of Presence units to stretch continued to large amplitudes of stretch (Matthews & Stein, 1969; Hasan & Houk, 1975; Hulliger *et al.* 1977; Kakuda, 2000).

Morphological observation

The muscle spindles were isolated by teasing with fine needles and then placed on a glass slide. Teased preparations of muscle spindles were examined using a light microscope (Eclipse E800; Nikon Corp., Tokyo, Japan) and were photographed (C5810; Hamamatsu Photonics KK, Shizuoka, Japan). Primary and secondary endings were identified based on their different locations, their axon diameters, and appearances of the muscle spindles. For old rats, differences in their location were used in most instances because their appearance and axon diameters apparently changed with age. We observed their morphological appearance and quantitatively assessed measured morphometrics including the ending length (longitudinal extent of unmyelinated endings that lie along fibres), number of bands for each intrafusal fibre, and axon diameters of spindle afferents using image processing software (ImageJ 1.34s; National Institutes of Health, USA). The diameters of Ia and II afferents were measured near the muscle spindle entry.

Data analyses

The electrophysiological data of muscle spindle afferents were pooled in each age group. For data of morphometrics of primary and secondary endings, the mean value was calculated for each muscle spindle; then the mean value for

each age group was obtained. All means are given along with the standard deviation (s.d.). The effects of ageing and differences between muscle spindle ending types were evaluated using one-way analysis of variance with Sheffe's *post hoc* analysis. The criterion for accepting statistical significance was $P < 0.05$.

Results

In all, 197 spindle afferents were analysed: those of 82 young rats, 44 middle-aged rats, and 71 old rats. Two types of response (presence or absence of slowing discharge) during the release phase under ramp-and-hold stretch were observed in all age groups. The Presence units were recorded for 18 afferents in young rats, 20 afferents in middle-aged rats, and 60 afferents in old rats. Absence units were recorded in 64 afferents in young rats, 24 afferents in middle-aged rats, and 11 afferents in old rats.

Axonal conduction velocity

Figure 3A shows histograms of the CV distributions for afferents in three age groups. The respective CVs of Presence units in young, middle-aged and old rats were 34–53 m s⁻¹, 33–50 m s⁻¹, and 21–50 m s⁻¹. Those of Absence units in young, middle-aged and old rats were 52–74 m s⁻¹, 53–66 m s⁻¹, and 35–50 m s⁻¹, respectively. The CV distribution showed two peaks in young rats, although they overlapped slightly; also, a dividing point at 50 m s⁻¹ was apparent for middle-aged rats. In contrast, Presence and Absence units were widely overlapped in old rats. The CVs of both Presence and Absence units in old rats were significantly lower than those in young and middle-aged rats, but those of Absence units differed to a greater degree. Figure 3B shows that the mean

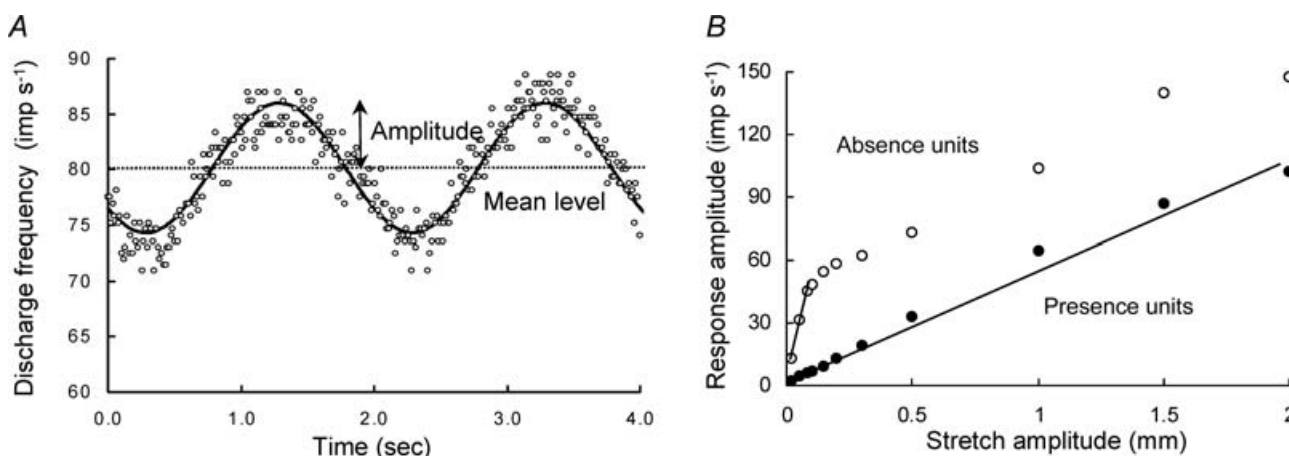


Figure 2. Measurement of the response of muscle spindle afferents to sinusoidal stretch

A, instantaneous discharge of muscle spindle afferents (o) and the fitted sine curve (thick line). B, respective relations between the amplitudes of stretch and the response of Presence and Absence units.

diameters of both Ia and II afferents in old rats (3.5 ± 0.7 and $2.3 \pm 0.7 \mu\text{m}$, respectively) were significantly thinner than those of young (5.7 ± 0.6 and $3.2 \pm 0.7 \mu\text{m}$) and middle-aged rats ($5.4 \pm 0.7 \mu\text{m}$ and $3.0 \pm 0.6 \mu\text{m}$).

Dynamic response during ramp-and-hold stretch

The DI increased concomitant with the increased stretch velocity. The plots of DI (log) versus stretch velocity (log) were well fitted by straight lines (Fig. 4A). The

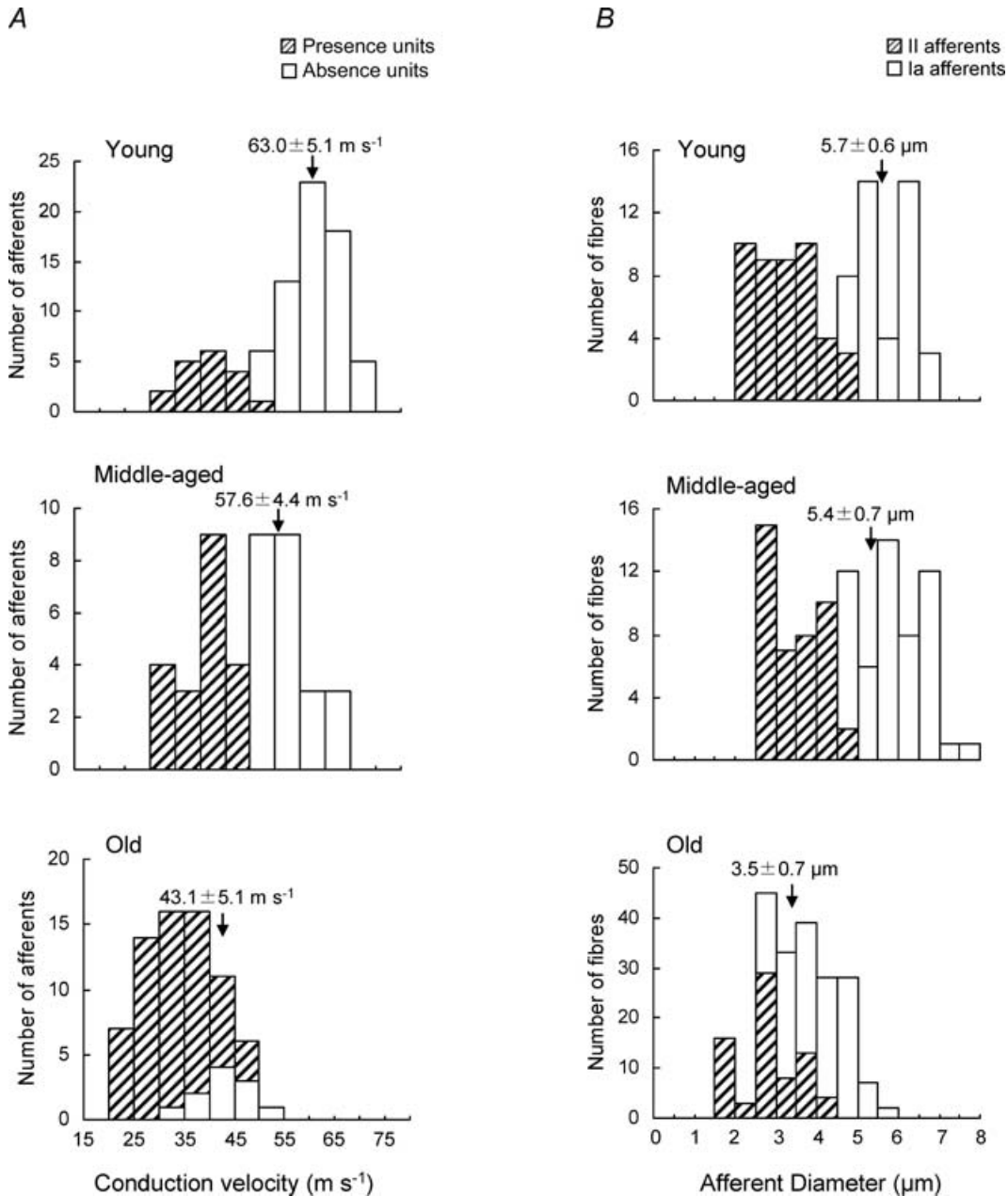


Figure 3. Distribution histogram of conduction velocity of muscle spindle afferents (A) and of the diameters of Ia and II afferents near muscle spindle entry (B) in young, middle-aged and old rats

A, the distribution of CV in young and middle-aged rats shows two peaks, although the CVs of Presence and Absence units in old rats are mostly overlapped. B, the distribution of diameters of 40 spindle Ia afferents and 45 II spindle afferents in young rats, 52 spindle Ia afferents and 43 II spindle afferents in middle-aged rats, and 128 spindle Ia afferents and 73 II spindle afferents in old rats. The mean diameter of Ia afferents in old rats was much less than those of young and middle-aged rats. These morphological data suggest the decrease in CV of old rats. The values of CV and diameters (means \pm s.d.) of Absence units are indicated in the figure.

slopes of the regression lines (exponent coefficients), which indicated dynamic sensitivity, for Absence units of muscle spindles in old rats ($0.45 \pm 0.07 \text{ imp s}^{-1} \text{ per mm s}^{-1}$) were significantly smaller than those for muscle spindles in young and middle-aged rats (0.53 ± 0.05 and $0.51 \pm 0.05 \text{ imp s}^{-1} \text{ per mm s}^{-1}$, respectively), but those of Presence units did not differ (Fig. 4B–D). The exponent coefficients of Presence and Absence units in old rats were overlapped over a wider range ($0.3\text{--}0.5 \text{ imp s}^{-1} \text{ per mm s}^{-1}$) than for young and middle-aged rats ($0.45\text{--}0.5 \text{ imp s}^{-1} \text{ per mm s}^{-1}$).

Figure 5 shows DI at 20 mm s^{-1} plotted against CV for the three age groups. The Presence units of young and middle-aged rats were distributed around lower DI ($45.3 \pm 18.9 \text{ imp s}^{-1}$ and $46.9 \pm 17.3 \text{ imp s}^{-1}$, respectively) and slower CV ($42.5 \pm 5.3 \text{ m s}^{-1}$ and $40.5 \pm 4.6 \text{ m s}^{-1}$, respectively). The Absence units of

young and middle-aged rats were distributed around higher DI ($125.8 \pm 39.5 \text{ imp s}^{-1}$ and $128.5 \pm 30.6 \text{ imp s}^{-1}$, respectively) and faster CV ($63.0 \pm 5.1 \text{ m s}^{-1}$ and $57.6 \pm 4.4 \text{ m s}^{-1}$, respectively). In contrast, as shown in Fig. 5C, both Presence and Absence units in old rats were distributed around lower DI ($39.4 \pm 13.8 \text{ imp s}^{-1}$ and $85.3 \pm 53.7 \text{ imp s}^{-1}$, respectively) and slower CV ($33.6 \pm 6.3 \text{ m s}^{-1}$ and $43.1 \pm 5.3 \text{ m s}^{-1}$, respectively). The DI values of Absence units in old rats were significantly lower than those of young and middle-aged rats. Consequently, the exact point of difference between Presence and Absence units was indistinct despite the significant difference between the means of the Absence and Presence units in the old rats. Apparently, the DIs and CVs of Absence units were shifted toward those of Presence units. The decrease of DI in old rats was mainly attributable to the low peak frequency (PF). The discharge

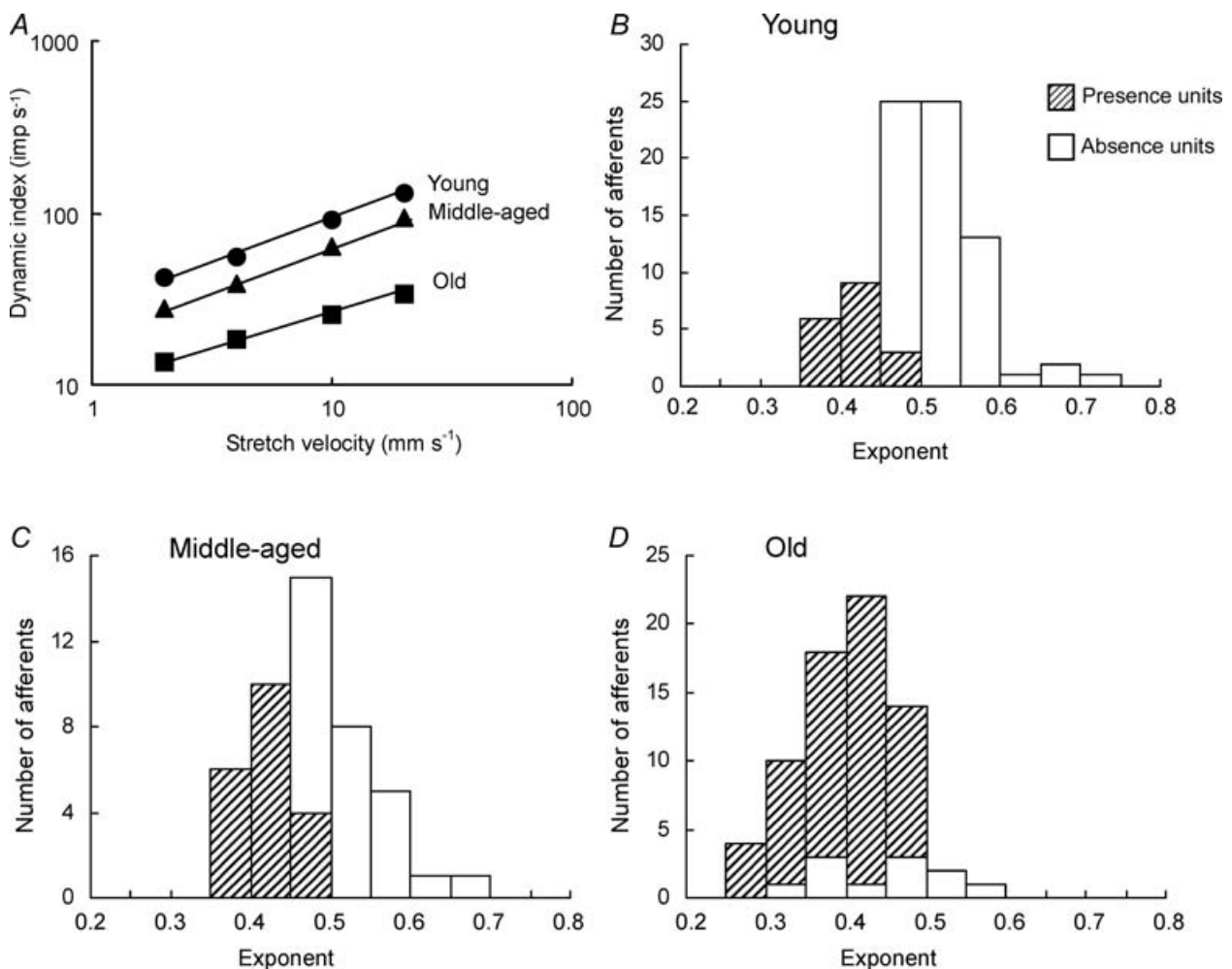


Figure 4. The representative relationship between DI (log) and stretch velocities (log), indicating the dynamic sensitivity (A) and the distribution of the slopes of the regression lines (exponent coefficients) indicating the dynamic sensitivity for Absence units and Presence units of muscle spindles in young (B), middle-aged (C), and old (D) rats

The distribution for Absence units in old rats is shifted markedly to the left.

frequency of muscle spindle afferents to ramp stretch rose abruptly to a peak value at the end of stretch in young rats (PF, $196.8 \pm 71.1 \text{ imp s}^{-1}$ at 20 mm s^{-1}), although they rose slowly in old rats (PF, $138.6 \pm 45.6 \text{ imp s}^{-1}$). The discharge frequency of muscle spindle afferents at 0.5 s after completion of ramp stretch was similar for young ($92.3 \pm 32.8 \text{ imp s}^{-1}$) and old rats ($91.1 \pm 29.6 \text{ imp s}^{-1}$).

Effects of succinylcholine

In all, 80 afferents (30 afferents from young rats, 28 afferents from middle-aged rats, and 22 afferents from old rats) were recorded for a ramp-and-hold stretch before and after the i.v. injection of SCh ($200 \mu\text{g kg}^{-1}$), with the intention of testing for increased dynamic response. Most units indicated an increased discharge at 30 s after the i.v. dose of SCh and reached a peak value at 60–120 s, with full recovery at 1200 s. After i.v. SCh, Absence units (Fig. 6A) showed slightly slowing discharge during the release phase and a great increase in DI ($18.9 \pm 14.1 \text{ imp s}^{-1}$), whereas Presence units (Fig. 6B) showed a low increase or decrease in DI ($2.2 \pm 3.9 \text{ imp s}^{-1}$) in young rats. A significant difference was found between the mean values of DI before and after SCh injection for Absence units ($P < 0.001$) in young and middle-aged rats. Furthermore, SCh injection enables clearer classification of spindle afferents into Absence units and Presence units (Fig. 7A and B). However, in old rats, spindle afferents were not classifiable into Absence units and Presence units because of the small increase of DI after SCh injection (Fig. 7C).

Response to sinusoidal stretch

Of the 197 spindle afferents studied previously under ramp-and-hold stretch, 24 were analysed using sinusoidal stretch (5 afferents from young rats, 5 afferents from middle-aged rats, and 14 afferents from old rats). Units with Presence or Absence under ramp-and-hold stretch corresponded to units with a broader linear range or units with a narrow linear range under sinusoidal stretch. The Absence units of all age groups increased nonlinearly to graded amplitude of sinusoidal stretch. That linear increase was limited to amplitudes of less than 0.08 mm (linear range, 0.02–0.08 mm) in all age groups. The Presence units of all age groups increased linearly with amplitude to 2 mm (linear range, 0.02–2 mm).

Morphological observation

For silver analyses, 121 spindles were teased from 10 medial gastrocnemius muscles that had been used in electrophysiological experiments: 34 spindles from three muscles of young rats, 35 spindles from three muscles of middle-aged rats, and 52 spindles of old rats. Figure 8A shows a representative complete muscle spindle of a young rat under a light microscope. The

Ia and II axons were readily distinguishable by their different diameters. Primary and secondary endings were readily distinguishable by their different locations and appearance in the muscle spindle. Terminations of primary endings innervated each intrafusal muscle fibre in the equatorial region; terminations of secondary endings innervated in the juxta-equatorial region. The bag and chain fibres were readily identified by their markedly different diameters. We were unable to determine bag fibre types (bag_1 , bag_2) with certainty, but terminals of primary

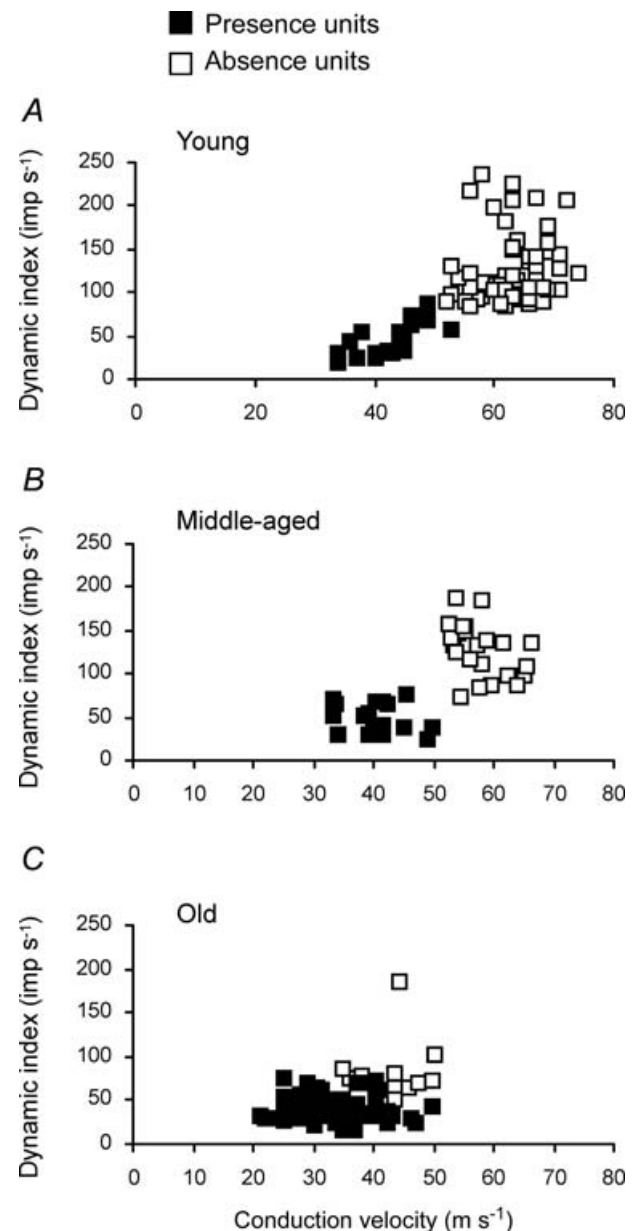


Figure 5. Dynamic index at 20 mm s^{-1} against conduction velocity for young (A), middle-aged (B), and old (C) rats
A and B, distinct populations of Presence and Absence units. C, both Presence and Absence units are distributed around the lower dynamic index and slower conduction velocity. Absence units are shifted toward Presence units in old rats.

afferent always supplied to at least one of the bag fibres in all age groups. Therefore, there might not be changes with ageing in the distribution of terminations on the different intrafusal fibres for either primary or secondary endings. The mean numbers of primary and secondary endings for each spindle did not differ among age groups. Most muscle spindles had one primary ending adjacent to one secondary ending (young, $76.5 \pm 4.7\%$; middle-aged, $76.2 \pm 15.8\%$; old, $76.3 \pm 9.0\%$). Some muscle spindles had one primary ending and two secondary endings (young, $14.6 \pm 4.9\%$; middle-aged, $9.2 \pm 10.1\%$; old, $12.6 \pm 11.6\%$) or no secondary ending (young, $8.8 \pm 0.4\%$; middle-aged, $14.6 \pm 5.9\%$; old, $11.1 \pm 6.0\%$).

Interestingly, the morphological appearances of primary endings in aged muscle spindles differed from those of the young muscle spindles, despite the similarity of the muscle spindles' overall features. Those of muscle spindles of young and middle-aged rats were spiral (Fig. 8B and C), whereas those of old rats showed tapered and irregular configurations rather than spirals. We defined sensory endings as three types of muscle spindles in old rats: spiral (i.e. annulospiral, as shown in Fig. 9A), less spiral (i.e. flat and spiral, as shown in Fig. 9B), and non-spiral (i.e. not at all spiral, as shown in Fig. 9C). Most muscle spindle primary endings were spiral type endings in young and middle-aged rats. However, in old rats, the less spiral type and non-spiral type accounted, respectively, for

$46.3 \pm 0.5\%$ and $40.5 \pm 9.3\%$ for each muscle (Table 1). Furthermore, the mean length of 45 primary endings (longitudinal extent of unmyelinated endings that lie along fibres) in young rats was $167.1 \pm 32.8 \mu\text{m}$. That of 34 primary endings in middle-aged rats was $165.9 \pm 24.0 \mu\text{m}$. In contrast, 81 primary endings of old rats had mean length of $126.8 \pm 24.8 \mu\text{m}$ (Table 2). The primary endings of old rats were significantly shorter than those of young and middle-aged rats. Many less spiral or non-spiral type endings existed in old rats; consequently, the transverse bands for each intrafusal fibre were significantly fewer than for those of young and middle-aged rats (Table 2). Because of the irregular characteristics of secondary endings, it was not possible to make a quantitative assessment of appearances of muscle spindles of old rats, but most appeared to be similar to those supplied to young and middle-aged spindles.

Discussion

We demonstrated that most units sampled from old rats showed properties of secondary muscle spindles that are observed in young rats. Our light microscopic observations revealed that primary endings were changed into less spiral or non-spiral endings in old rats, but they still existed. These findings suggest that primary muscle spindle endings tend to become physiologically similar to secondary endings in old rats.

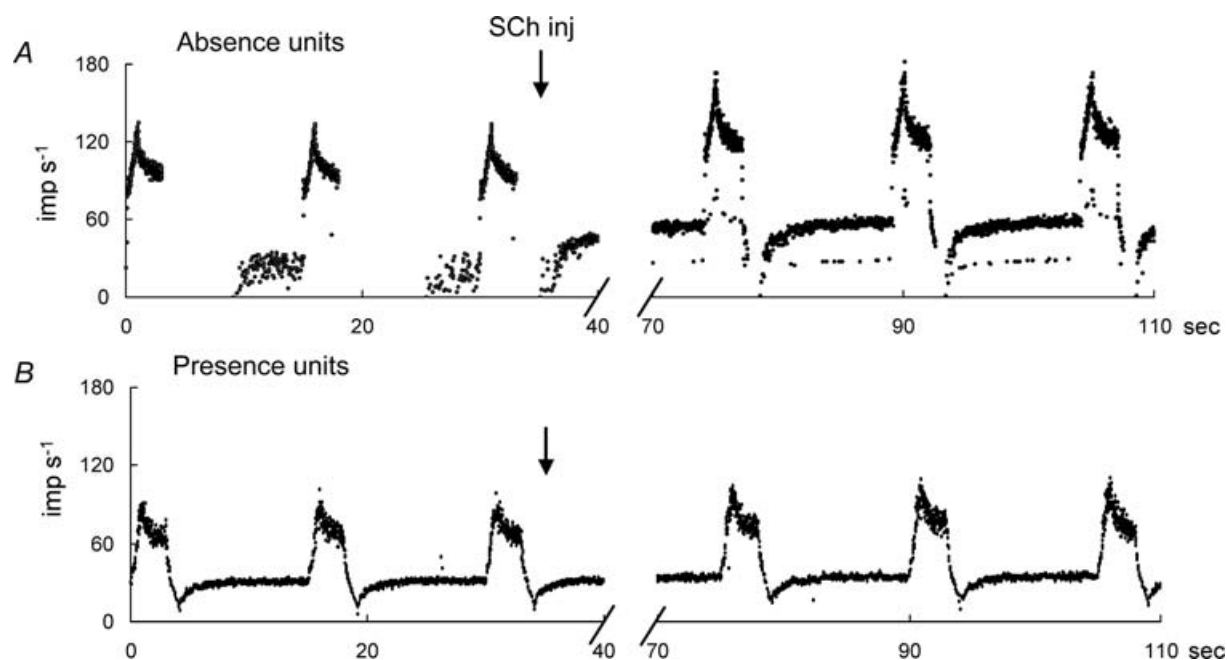


Figure 6. Representative responses to ramp-and-hold stretch before and after SCh injection ($200 \mu\text{g kg}^{-1}$)

A and B, representative responses of Absence and Presence units, respectively.

Classification of primary and secondary endings in old rats

Conduction velocity. The CV has long been used as a criterion for classifying muscle spindle afferents in cat (Matthews, 1963; Wei *et al.* 1986; Barker *et al.* 1986; Scott, 1990), rat (De-Doncker *et al.* 2003), and primate (Cheney & Preston, 1976). The present experiments classified muscle spindle afferents into Absence and Presence units as two peaks in young and middle-aged rats (results presented herein suggest that our Absence units corresponded to primary endings and our Presence units

to secondary endings), whereas the CVs in old rats were lower for both Absence units (31.7%) and Presence units (20.9%) than those of young or middle-aged rats. These results were supported by the present morphological data, which show decreased diameters of Ia and II afferents in old rats, and by data obtained by many previous physiological and morphological studies (Chase *et al.* 1992; Bergman & Ulfhake, 1998; Pannese *et al.* 1998; Sugiura & Kanda, 2004). Chase *et al.* (1992) described that an age-dependent decrease in CV was related to decreased axon diameter and thinning of the myelin sheath. Our

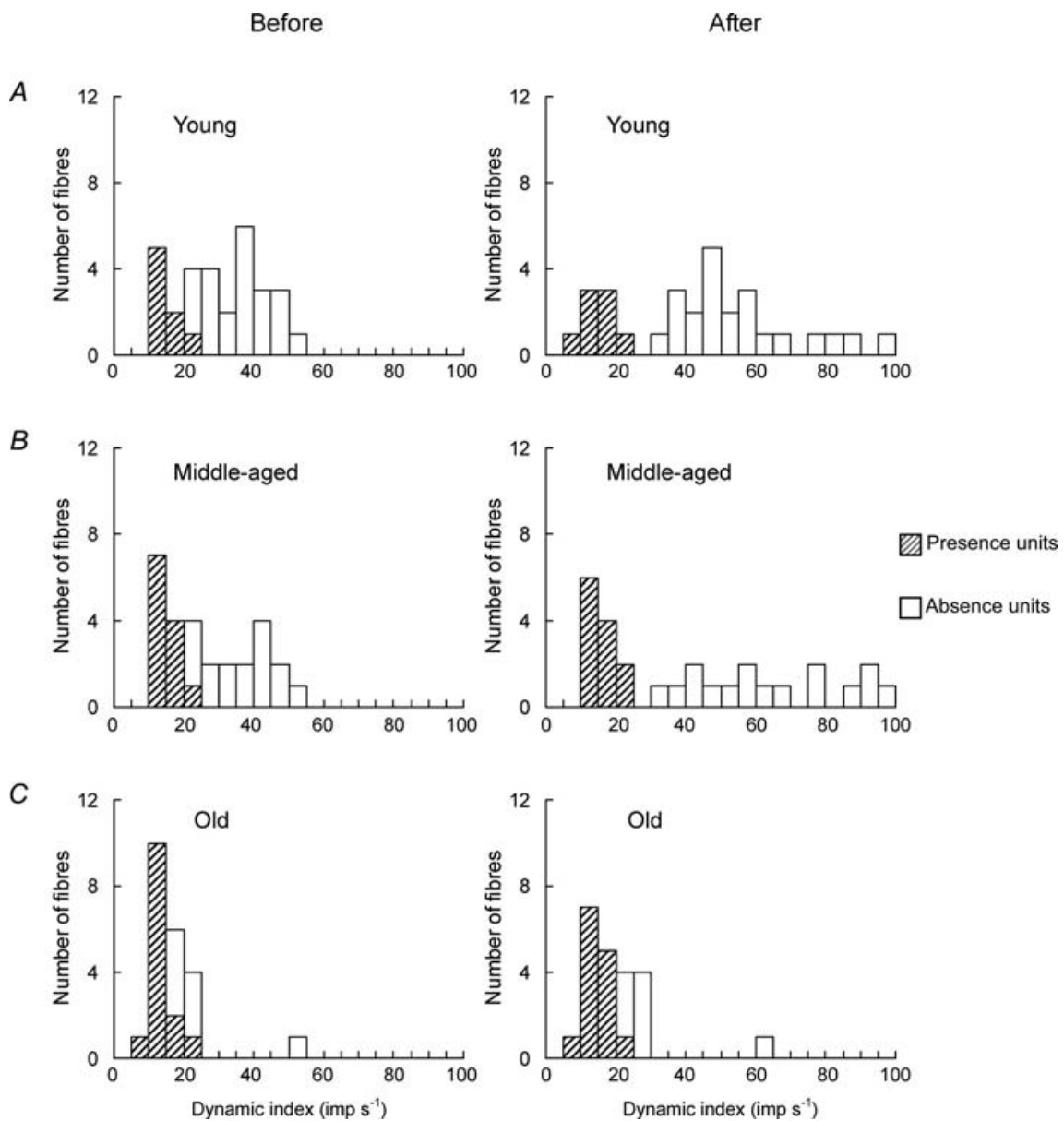


Figure 7. Distribution histograms of the values of DI of Absence and Presence units before and after SCh injection in young (A), middle-aged (B), and old (C) rats
The responses show no distinction in old rats after SCh injection.

previous experiments (Sugiura & Kanda, 2004) revealed that the decrease in CV of motoneurons with ageing was greater for CV of motoneurons belonging to the fast motor units than for those of motoneurons belonging to the slow motor units. The difference between these two groups was therefore small. Evidence also indicates loss of the dorsal root ganglion neurons (DRG) and selective cell body atrophy among myelinated primary afferents with ageing (Bergman & Ulfhake, 1998; Pannese *et al.* 1998).

Dynamic response. The present experiments showed that the dynamic sensitivity of muscle spindle primary endings was decreased in old rats: the peak frequency during the dynamic phase of muscle stretch was much lower than that in young and middle-aged rats. The peak discharge frequency was determined by both ionic and mechanical properties of intrafusal muscle fibres (Boyd

& Smith, 1984). Fischer & Schafer (2000) revealed that both the impulse activity and the sensitivity to stretch decreased in a high Ca^{2+} solution and increased in a low Ca^{2+} solution. Matthews (1964) suggested that such a pattern of sensory discharge might occur as a result of visco-elastic properties of the spindle receptor. Direct observation of spindles revealed that the bag fibres behave in a visco-elastic manner, whereas the chain fibres appear to be almost elastic (Boyd, 1966; Gladden, 1972; Cooper & Gladden, 1974; Boyd & Ward, 1975). The primary sensory ending is wrapped around both the viscous bag and the elastic chain fibres, whereas the secondary sensory ending lies principally around the elastic chain fibres (Banks *et al.* 1982; Boyd, 1985). Therefore age-related changes in the ionic and mechanical properties of intrafusal fibres might engender decrease of the dynamic response of the primary endings. The injection of SCh

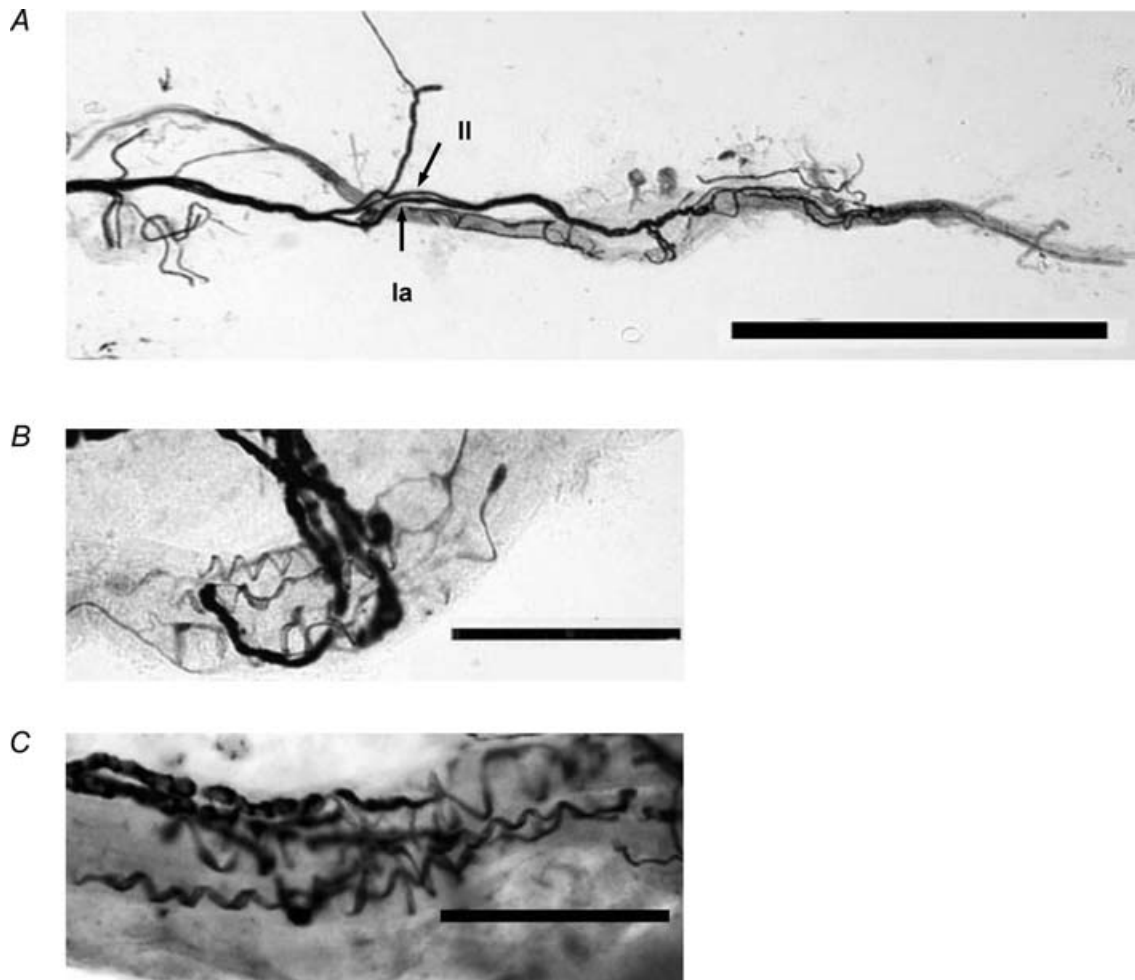


Figure 8. Example of teased silver-impregnated muscle spindles viewed under a light microscope

A, all silver-impregnated spindles isolated from the medial gastrocnemius muscles of a young rat. Arrows, respectively, indicate Group I and Group II. *B* and *C*, equatorial regions of muscle spindles in a young rat and a middle-aged rat, respectively. The primary endings are spiral. Scale bars indicate 1 mm in *A*, and 100 μm in *B* and *C*.

Table 1. Numbers of muscle spindle types from medial gastrocnemius muscles of young, middle-aged, and old rats

Age groups	No. of spindles	Spiral	Less spiral	Non spiral
Young no. 1	11	11	0	0
Young no. 2	12	12	0	0
Young no. 3	11	11	0	0
Middle-aged no. 1	10	10	0	0
Middle-aged no. 2	12	11	1	0
Middle-aged no. 3	13	13	0	0
Old no. 1	13	2	6	5
Old no. 2	11	2	5	4
Old no. 3	13	0	6	7
Old no. 4	15	3	7	5

Most muscle spindle primary endings in young and middle-aged rats are spiral type endings. Many muscle spindles in old rats are of less-spiral and non-spiral types.

helped clarify the classification of spindle afferents in young and middle-aged rats, but it did not allow two group endings to be distinguished clearly because of the small increase in the dynamic response of Absence units in old rats. These results also suggested that mechanical properties of nuclear bag fibres might change with ageing because the excitation by SCh of the spindle primary endings has been known to cause intrafusal contraction of the two nuclear bag fibres (Gladden, 1976; Dutia, 1980; Carr & Proske, 1996). To our knowledge, however, no study has examined age-related changes in the ionic and mechanical properties of intrafusal fibres. Another reason for the decline of dynamic response in old rats is considered to be the change in the distribution of primary and secondary terminations on the different intrafusal fibres. We also tested a similar parameter described by Taylor *et al.* (1992) to examine age-related changes in the distribution pattern of units (data not shown), but our data showed no clear distribution in old rats because of the few samples that were used. More details of age-related changes in ionic and mechanical properties of intrafusal fibres, along with details of the distribution of terminations on different intrafusal must be obtained in future studies.

Response to sinusoidal stretch. The linear range of Presence units was broader than that of Absence units for all age groups. These results accord with previous studies, in which secondary endings showed a broader linear range than primary endings in rat (De-Doncker *et al.* 2003), cat (Matthews & Stein, 1969; Hasan & Houk, 1975; Hulliger *et al.* 1977), and human (Kakuda, 2000). Units with Presence or Absence under ramp-and-hold stretch corresponded to units with a broader linear range or units with a narrower linear range under sinusoidal stretch, suggesting that our Absence units corresponded

to primary endings and that our Presence units to secondary endings, as previously described. Therefore, a linear range during sinusoidal stretch and two types of response (presence or absence of slowing discharge) during the release phase under ramp-and-hold stretch might be the only criteria for classification of primary and secondary endings, even in the aged rat model. On the other hand, the population of Absence units was far smaller among old rats (11/71) than among young (64/82) or middle-aged rats (24/44), thereby suggesting one of two possibilities. Primary endings disappear preferentially, or primary endings change to show slowing discharges similarly to secondary endings during the release phase under ramp-and-hold stretch in old rats. Therefore, such criteria as the linear range during sinusoidal stretch and two types of response (presence or absence of slowing discharge) during the release phase under ramp-and-hold stretch are not useful for classifying primary and secondary endings in muscle spindles of old rats.

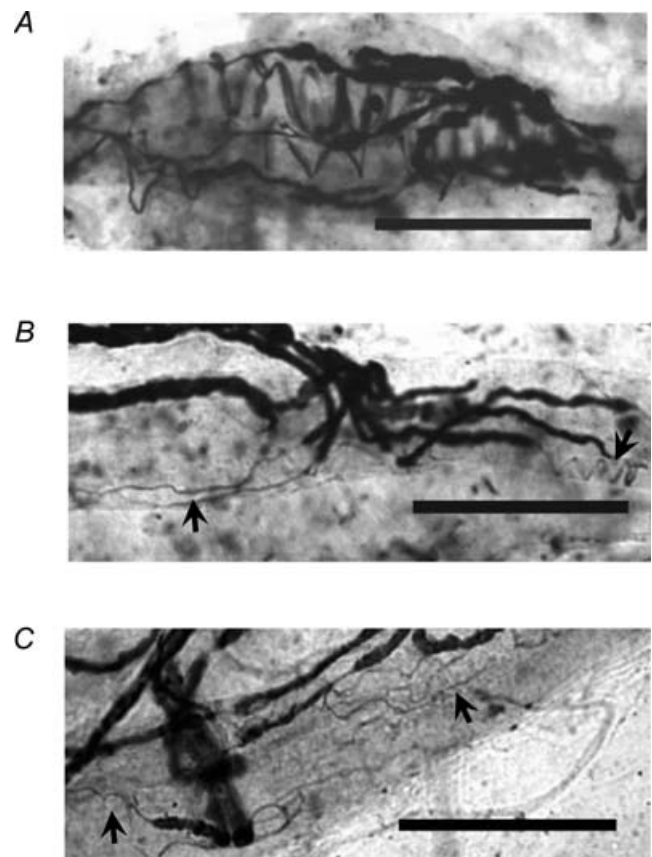


Figure 9. Equatorial regions of muscle spindles in an old rat Primary endings are three muscle spindle types in old rat: as spiral (A), less spiral (B), and non-spiral (C). Arrows in B and C indicate tapered and irregularly shaped sensory terminals. See text for type definitions. Scale bars indicate 100 μ m in A, B and C.

Table 2. Mean length of endings and mean number of transverse terminal bands

Age groups	No. primary endings analysed	Mean ending length \pm S.D. (μm)	Mean no. transverse bands \pm S.D.
Young	45 (0)	167.1 \pm 32.8	9.7 \pm 2.5
Middle-aged	34 (1)	165.9 \pm 24.0	8.8 \pm 2.2
Old	81 (65)	126.8 \pm 24.8*	3.4 \pm 2.6*

The mean length of endings and mean number of bands for each intrafusal fibre are significantly less in primary endings of old rats (* $P < 0.01$). Numbers of less-spiral and non-spiral endings are shown in parentheses.

Age-related morphological changes of sensory nerve endings

One study reported that there were 28 spindles in rat medial gastrocnemius muscle (Arendt & Asmussen, 1974). However, 17 spindles were identified in the rat medial gastrocnemius muscle that we counted by serial section (data not shown). A study of the number of spindles in certain cat limb muscles (Barker & Chin, 1960) revealed considerable interindividual variation. Therefore, we infer that the morphological differences found in the present experiments reflect general changes that occur in old rats.

Age-related morphological changes in peripheral nerves have been reported extensively: axonal atrophy (Ochoa & Mair, 1969; Knox *et al.* 1989; Chase *et al.* 1992), decreased internodal length (Lascelles & Thomas, 1966), demyelination (Knox *et al.* 1989; Adinolfi *et al.* 1991), and decline in motor nerve terminal branches and acetylcholine receptor rich areas of the neuromuscular junction (Oda, 1984; Balice-Gordon, 1997). The present study provides evidence that degenerative changes also exist in sensory nerve endings in old rats. Immunohistochemical and fine structural studies revealed drastic changes in the structures, not only of intrafusal muscle fibres but also of nerve endings in aged rat jaw muscle spindles (Winarakwong *et al.* 2004). Electron microscopic observations of them showed that closely packed myofibrils become attenuated; some sensory terminal profiles of bag fibres enlarge, and occasionally parts of the equatorial region of intrafusal fibres were devoid of nerve terminals. Moreover, the morphology of immunoreactive sensory terminals of old animals also displayed somewhat irregular and less crowded sensory endings. The present morphology results for primary endings of old rats are consistent with those results. Other light microscopic observations indicated degenerative changes in aged human intrafusal muscle fibres and their motor nerve endings (Swash & Fox, 1972; but see also Winarakwong *et al.* 2004).

Several physiological and morphological studies of regenerated muscle spindles after nerve injury have shown that the responses of the regenerated spindle afferents

to stretch were restored gradually to normal spindles during recovery, although all their endings were abnormal, showing tapered or irregular configurations rather than a spiral form (Quick & Rogers, 1983; Barker *et al.* 1986; Banks & Barker, 1989; Barker & Scott, 1990). However, the mechanism of that recovery in the response of muscle spindle afferents to stretch is less clear. Hyde & Scott (1983) suggested that the return to normality is probably the result of the pacemaker thresholds of the afferents gradually regaining their normal levels. Nevertheless, the present study showed that age-related changes of muscle spindle physiological properties are attributable to morphological changes of the terminals in old rats. The physiological findings merely suggested two possibilities: preferred dropout of the primary endings occurred in muscle spindles of old rats; alternatively, their physiological properties change to secondary-ending-like response. Additional morphological findings revealed that the primary endings exist in muscle spindles of old rats, therefore supporting the latter possibility. The innervated extent of sensory endings on intrafusal fibres reflects a capacity for depolarization of sensory endings because the stretch of intrafusal fibres mechanically deforms the sensory terminals, thereby altering the ionic permeability of the sensory terminal, which in turn depolarizes the terminal (Hunt *et al.* 1978). Consequently, it is strongly suggested that the present morphological results, which lack longitudinal innervated length and transverse banding, are related to reduction of dynamic responsibilities of the primary endings.

McCloskey (1978) reported that the discharges of primary endings are dependent on information about changes in muscle length and velocity, whereas the discharges of secondary endings are mainly dependent on information about changes in muscle length. For that reason, results of our study support the observation that the difference between young and old people in velocity of sway is greater with dynamic posturography than with static posturography (Baloh *et al.* 1994). Alteration of ionic conditions of peri-sensory nerve endings should also be investigated. Such studies will further elucidate age-related motor changes.

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Acknowledgements

We are grateful to Dr S. Nomoto for critical reading of the manuscript.