博士（人間科学）学位論文

Age-related Physiological and Morphological Changes of Muscle Spindles in Rats

加齢に伴うラット筋紡錘の機能的・形態的変化

２００７年７月

早稲田大学大学院　人間科学研究科

金　祉希

Kim, Gee Hee

研究指導教員：　鈴木　秀次　教授

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to many people for their contribution in the preparation of this thesis. My deepest gratitude goes to Professor Shuji Suzuki for his guidance and continuous encouragement throughout my undergraduate and graduate study. If it were not for his generosity this thesis would have never been written. Extreme thanks are expressed to other committee members, Drs. Tetsuo Fukunaga, Terumasa Komuro, and Kazuhiko Imaizumi. Their thoughtful comments and suggestions definitely helped in elaboration of this dissertation. Special appreciation is expressed to Dr. Kenro Kanda for his academic expertise, generosity, and his limitless patience in helping me during my academic activity. I am very fortunate to have worked with him. I also express my appreciation to other committee members, Dr. Shigeki Nomoto, Emi Nomoto, Seiko Asaki, Narumi Kojima, Miki Aoki, Shozo Suzuki, and Shigenobu Matsumoto for their continuous encouragement and support while working on this thesis. My appreciation extends to each member of Suzuki Laboratory at Waseda University, Japan, for his/her favorable support. Finally, I would like to thank my family for their generous support and continuous encouragement.

TABLE OF CONTENTS

CHAPTERS

LISTS OF FIGURES AND TABLES

Figures

Tables

ABSTRACT

Age-related physiological and morphological changes of muscle spindles were examined in rats (male Fischer 344/DuCrj: young, 4-13; middle-aged, 20-22; 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) or sinusoidal stretch (amplitude, 0.05-1.0 mm; frequency, 0.5 Hz). Respective conduction velocities (CVs) were measured. After electrophysiological experimentation, the muscles were dissected. The silver-impregnated muscle spindles were teased and then analyzed 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.

CHAPTER I

INTRODUCTION

The number of elderly who need long-term care is increasing, while the birthrate declines and the proportion of elderly increases. Regular physical activity by the elderly makes an important contribution to the maintenance of good health, reduces the impact of disease, and delays disability. On the other hand, it is known that various motor functions decline with aging; e.g. a decrease in gait stability (Hausdorff *et al.*, 1997; Hurley *et al.*, 1998), slowing of reaction movements (Welford, 1988), a loss of fine coordination, and an increase in postural sway (Overstall *et al.*, 1977; Alexander, 1994). 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 these reasons, we focused on age-related changes of muscle spindles, probably causing decline of postural and locomotion control in elderly.

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 old rats. Furthermore, recent morphological studies have revealed that sensory nerve endings which are immunoreactive to PGP9.5 showed degeneration with aging (Yamamoto *et al.*, 2003; Winarakwong *et al.*, 2004). However, little is known about actual effects of aging 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 (CV) (Lafratta & Canestrari, 1966; Dorfman & Bosely, 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 aging 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 fibers (Verdu *et al.*, 2000).

The purpose of the present study was to investigate age-related changes in physiological properties and structure in muscle spindles. In this respect, physiological and morphological studies were conducted in three age-group rats (young:

4-13.5 months of age; middle-aged: 20-22 months of age; old: 28-31 months of age). In the physiological study, four experiments were conducted, each of which assessed: 1) CV in muscle spindle afferents, 2) afferent discharge response of muscle spindles to passive ramp-and-hold stretch, 3) afferent discharge response of muscle spindles following injection of muscle depolarizing drug succinylcholine (SCh), and 4) afferent discharge response of muscle spindles to passive sinusoidal stretches. In the morphological study, the morphology of muscle spindle sensory endings was examined with the teased silver-impregnated muscle spindles under a light microscope.

Overview

Following Chapter 1 as an introduction, Chapter 2 provides a comprehensive account of numerous details in the structure and physiological properties of the muscle spindle. Then, Chapter 3 concerns physiological experiments 1, 2, 3 and 4. Next, Chapter 4 concerns the morphological experiment. Finally, Chapter 5 presents a general discussion about the findings from the physiological study and the morphological study.

CHAPTER II

REVIEW OF LITERATURE

The muscle spindle was first identified in muscle by Kolliker for the frog (1862) and by Kuhne for the mammal (1863). They interpreted that the muscle spindle consists of both nervous and muscular elements. In 1894, Sherrington demonstrated that the muscle spindle received one or more myelinated afferent fibers and was proved to be a 'sensorial organ'. Subsequently, the muscle spindle has been studied extensively by a lot of morphologists and physiologists. The present chapter provides comprehensive account of numerous details in structure and physiological properties of the muscle spindle.

Structure of muscle spindle

Intrafusal muscle fibers

Boyd (1960) described difference between intrafusal muscle fibers which they might fall into two morphologically distinct classes. The two kinds of intrafusal muscle fibers were named 'nuclear bag fibers' and 'nuclear chain fiber' on the basis of the arrangement of nuclear in their central regions: the nuclear bag fibers is accumulation of 100 or more nuclei at the spindle equator underlying the terminals of the primary sensory ending and the nuclear chain fiber is a single row of nuclei in the equatorial region. Subsequently, the two nuclear bag fibers are themselves of two distinct types termed ‘bag_1 fiber’ and ‘bag_2 fiber’ because of the histochemical differences between them (Ovalle & Smith, 1972), or ‘dynamic bag fiber’ and ‘static bag fiber’ because of their differing mechanical properties (Boyd *et al*, 1975). Figure 2.1 are indicated diagrammatically the structure of a typical muscle spindle from a cat hindlimb muscle. Cat muscle spindle usually contains two relatively large nuclear bag fibers about 10 mm long and 25 μm in diameter, and three or five smaller nuclear chain fibers about 4 mm long and 12 μm in diameter. In rat muscle spindles, previous histochemical studies have been demonstrated that muscle spindle usually contains two bag fibers and one or two chain fibers (Kucera *et al*., 1978; Takamatsu, 2003). The muscle spindle is contained within a thick connective tissue capsule which expands in its center to form a fluid-filled space, giving the structure its characteristic fusiform appearance. Boyd (1962) found that chain fibers of the cat spindles generally terminated within the capsule, while those of the rat spindles reach beyond capsule limits without important attachment. There is also ultrastructural observation, particularly outer capsule, inner capsule, and fusimotor endings, of muscle spindles in the tenuissimus muscle of the Chinese hamster by scanning electron microscopy (Desaki, 2001).

Motor innervation

All spindles receive fusimotor innervation from γ motoneurons in the spinal cord. The existence of a different motor ending was established by degeneration experiments by Boyd & Davey (1962). Barker *et al* (1970) has subsequently described the morphological differences between plate and trail endings by using light and electron microscopes. The γ motoneurons were also subdivided using physiological techniques into 'dynamic' and 'static'. Dynamic and static fusimotor axons were originally distinguished by their actions on the group Ia discharge during stretch of the muscle (Matthews, 1962). This physiological difference is reflected in differences in their anatomical termination on the intrafusal muscle fibers. The dynamic γ axons innervate only the dynamic bag$_1$ fiber, whereas the static γ axons innervate either the static bag$_2$ or chain fibers alone, or both of them (Fig.2.1).

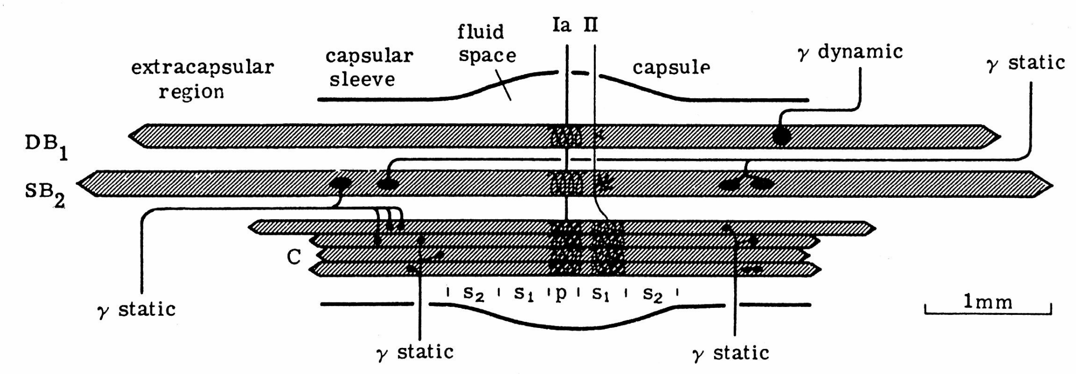

Figure 2.1. Diagram of structure and innervation of a typical muscle spindle from a cat hindlimb muscle. (from Boyd, 1980)

Sensory innervation

Ruffini (1898) first made a detailed study of the appearance of the nerve terminals within the spindle using gold chloride impregnation. He found two kinds of endings, which are supplied, respectively, by Ia and II fibers: primary and secondary endings. Figure 2.2 shows a silver stained muscle spindle teased from the peroneus longus muscle of the cat. The most common arrangement is for there to be one primary ending and one secondary ending adjacent to it. Primary endings consist of spiral or annular terminations on each of intrafusal muscle fibers of all three types in the equatorial region, whereas secondary endings have extensive irregular spiral or annular terminations on chain fibers and some spray-like terminations on bag fibers in the juxta-equatorial region. Figure 2.3 indicates the center of muscle spindle which has been stained with silver. It was isolated from the peroneus brevis muscle of the cat. The appearance of primary endings of rat muscle spindles was similar to those obtained in the cat (Takamatsu, 2003). Adal & Barker (1962) demonstrated in cat rectus femoris that the diameters of Ia was larger than those of II afferents which was 6-16 μm and 4-10μm, respectively.

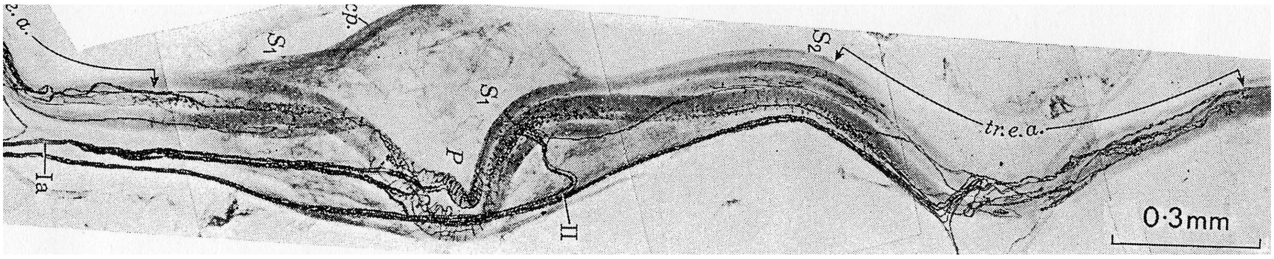

Figure 2.2. A nearly complete spindle isolated from cat. (from Barker, Stacey & Adal, 1970) *P*: primary ending, *S*: secondary ending, *cp*: capsule, *tr.e.a*: trail ending, *Ia* and *II*: group Ia and II afferents fibers respectively.

Figure 2.3. Typical primary endings of equatorial region in a cat spindle. (from Barker, 1967)

Physiological properties of spindle afferents

The classical distinction of receptor afferents

Matthews BHC (1933) was the first to record the discharge of single afferents from receptors in muscle and distinguished patterns of discharge in muscle spindle and Golgi tendon afferents. Both of muscle spindle and Golgi tendon endings increased their firing rate to muscle stretch, but the muscle spindle endings had a lower threshold than the Golgi tendon endings and often fired spontaneously in the absence of any external stretch at all. The more crucial difference between them was seen in their responses to an electrical muscle twitch. As shown in Fig.2.4, the discharge of muscle spindle endings abolished at the onset of the twitch, whereas the Golgi tendon afferents increased their rate of firing. This is exactly the behavior predicted for muscle spindle receptors and Golgi tendon organs. The muscle spindles, lying in parallel with the extrafusal muscle fibers, would be slightly slacken by the extrafusal contraction and reduce their discharge. The Golgi tendon organs, however, lie in series with the extrafusal muscle fibers and were thus excited by the twitch.

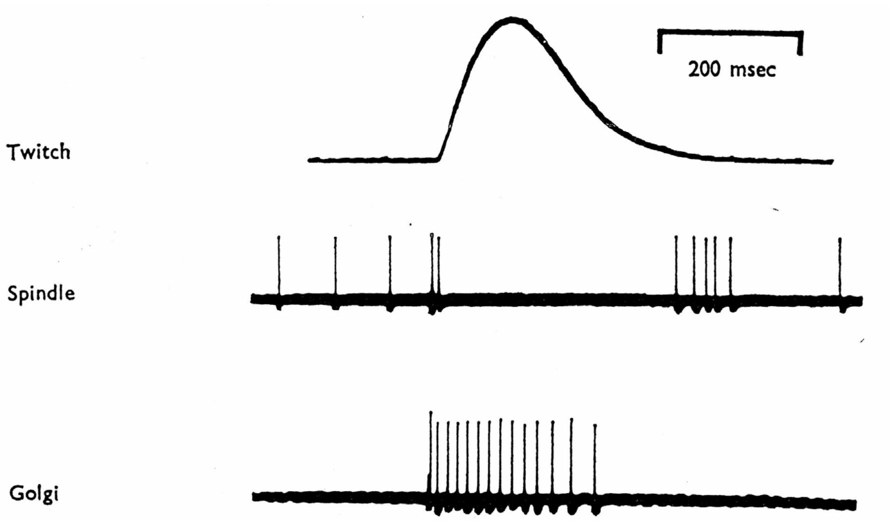

Figure 2.4. Contrasting responses of a spindle ending and a tendon organ during a twitch contraction. (from Matthews, 1972)

Conduction velocity of Ia and II afferent fibers

In 1954, Hunt made the first systematic study of primary and secondary endings on the basis of the CVs of their afferent fibers. Figure 2.5 shows the distribution of CVs of the afferent fibers. The distribution appeared bimodal, thus fitting in with the idea that there are two kinds of spindle afferent fiber. Boyd & Kalu (1979) also demonstrated in the cat hind limb that CV of Ia and II afferents fibers was 55-120 m/s and 20-65 m/s, respectively and these results related to the histological data, which the large fibers come from primary endings and the small fibers from secondary endings.

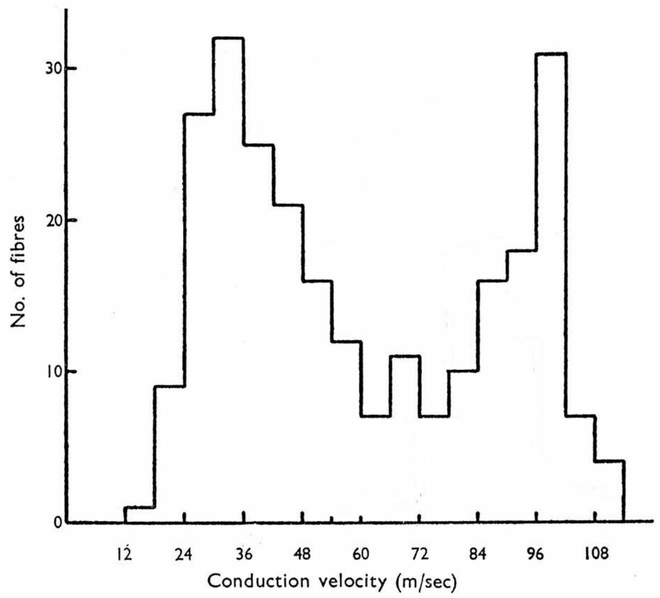

Figure 2.5. Distribution of the conduction velocities of the muscle spindle afferent fibers. (from Hunt, 1954)

Responses of primary and secondary endings to passive stretch

Matthews BHC (1933) noted that the muscle spindle discharged steadily when the muscle was at constant length (static response), but that a greater response was obtained during stretch and then the discharge adapting down to a steady level. The overshoot (dynamic response) was dependent on the rate of stretch of the muscle. Subsequently, Cooper (1961) discovered that primary endings were more sensitive to the rate of stretch than secondary endings. Figure 2.6 shows the response of primary and secondary endings to a passive ramp stretch. The dynamic response of the primary ending has several phases. First, at the onset of a ramp stretch the discharge frequency rapidly increase and rise to peak value at the end of the stretch. Then there is a rapid fall, followed by a phase of slow decay of frequency lasting several seconds. Crowe and Matthews (1964) measured the dynamic response which was the difference between the peak frequency at the end of the stretch and 0.5 sec later, and called ‘dynamic index’. Figure 2.7 illustrates the relationship between the dynamic index and the velocity of stretch for a primary and a secondary ending. For both, increasing the velocity of stretch increases the dynamic index, but for any particular velocity its value is consistently greater for the primary ending than for secondary ending.

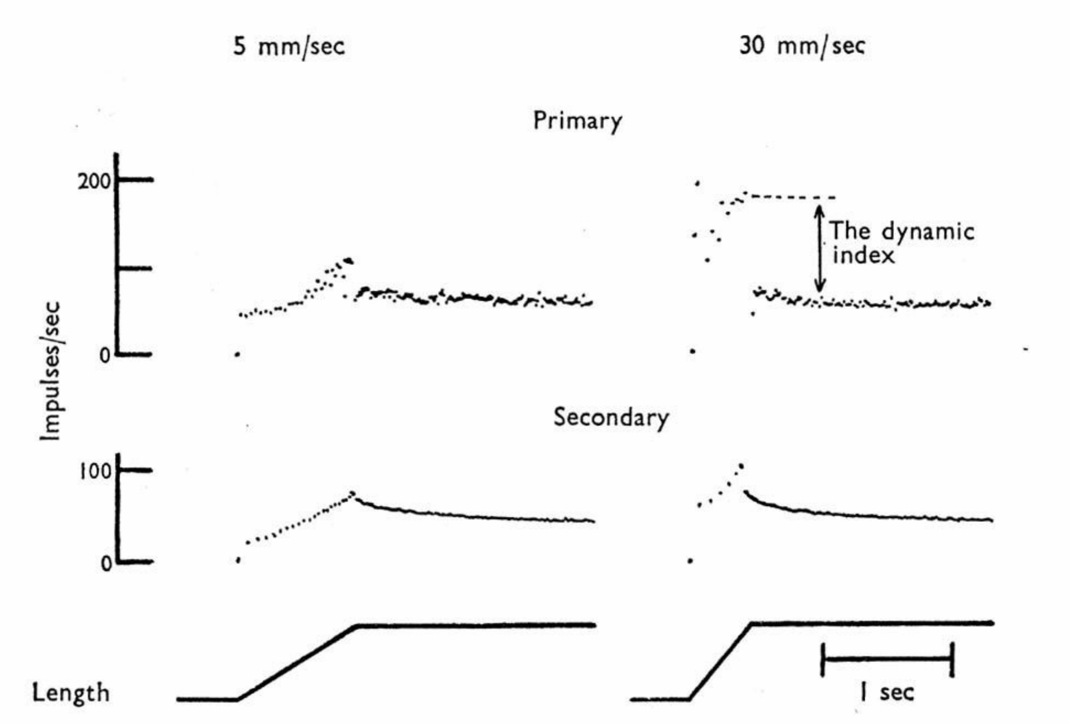

Figure 2.6. Comparison of the responses of de-efferented primary and secondary endings to ramp stretch of 5 mm/s and 30 mm/s. *upper row*: instantaneous discharge frequency of primary endings, *middle row*: instantaneous discharge frequency of secondary endings, *lower row*: displacement of stretch. (from Brown, Crowe & Matthews, 1965)

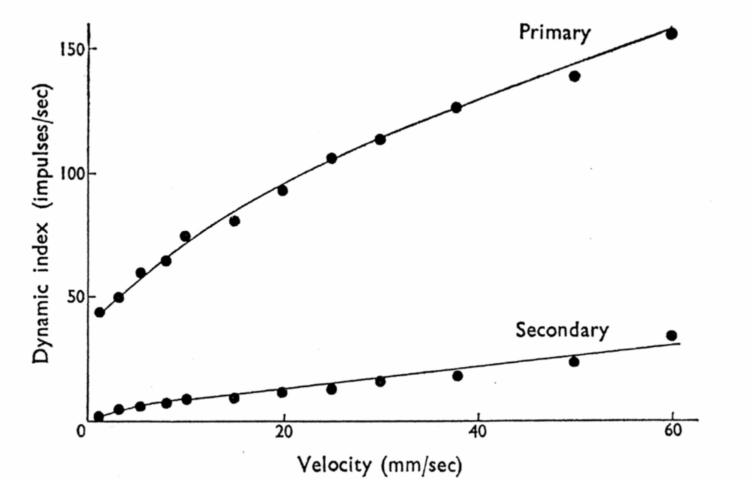

Figure 2.7. Relationship between the dynamic index and the velocity of stretch for a primary and a secondary ending in the sloeus muscle of cat. (from Matthews, 1963)

Some other differences between the responses of primary and secondary spindle endings to different types of muscle stretch are shown in Fig 2.8. Generally, primary endings are much more sensitive to ramp or sinusoidal stretch and to tendon tap, whereas secondary endings show little response to either type of input. During the release phase, however, primary endings show abrupt cessation of firing and secondary endings show lasting burst.

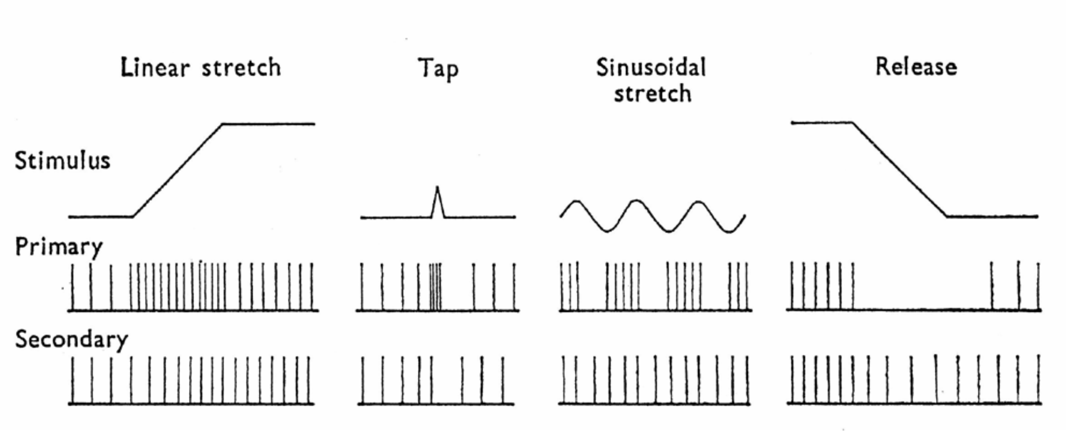

Figure 2.8. Diagrammatic comparison of the typical responses of primary and sensory endings to passive stretch in the absence of fusimotor activity. *upper row*: displacement of stretch, *middle row*: primary afferent activity, *lower row*: secondary afferent activity. (from Matthews, 1964)

CHAPTER III

PHYSIOLOGICAL STUDY

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. Miwa *et al* (1995) determined 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, they reported that 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 favor of secondary endings. Whether or not the current criteria, e.g. CV and response to stretch, are generally available for classification of primary and secondary endings in muscle spindles of old rats remains to be clarified.

The aim of the present physiological study is to examine whether or not the electrophysiological criteria are useful for classifying muscle spindles of old rats into primary and secondary endings. For this purpose, four experiments were conducted.

In Experiment 1, the CVs of sensory endings were measured. Numerous studies have classified primary and secondary endings based on their differences in CV (Hunt 1954; Cheney & Preston, 1976; Scott, 1990; De-Doncker *et al.*, 2003). Primary endings show faster CV, whereas secondary endings show slower CV. In Experiment 2, we examined the response to ramp-and-hold, in particular, dynamic response. Electrophysiological studies have demonstrated that primary endings show higher dynamic response, but secondary endings show lower dynamic response. (Cheney & Preston, 1976; Wei *et al.*, 1986; De-Doncker *et al.*, 2003). In Experiment 3, we attempted to classify spindle endings by their dynamic response change following injection of the muscle depolarizing drug SCh. The 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 Experiment 4, the response to sinusoidal stretches was examined. The pervious studies reported that primary endings show a narrower linear range on sinusoidal stretch, whereas secondary endings show a broader linear range on sinusoidal stretch (Matthews & Stein, 1969; De-Doncker *et al.*, 2003).

Experiment 1: Conduction velocity

Methods

Animals

Experiment 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 procedure

Rats were anesthetized using pentobarbital sodium (young, middle-aged, 50 mg/kg i.p.; old, 25 mg/kg i.p.). Adequate depth of anesthesia was monitored frequently by checking the pupil size and the flexion reflex to paw pinch. Supplementary injections (10 mg/kg) were administered when necessary. After the experiment, each rat was killed using an overdose of pentobarbital sodium (100 mg/kg).

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 hind limb 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 35-38°C. Rectal temperature was monitored and controlled to 36-38°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 (0.69-1.3 ml h^{-1}) to maintain adequate blood pressure.

Experimental protocol

Single afferent discharges of the muscle spindles in the gastrocnemius muscle were recorded from a finely split dorsal root (L4-L6). As shown in Fig 3.1, muscle spindles were distinguished from Golgi tendon organs by their different discharge behaviour during a twitch. Then we classified muscle spindle afferents into Absence and Presence units according to their response during the release phase (Fig.3.2), which showed either absence or presence of slowing discharge (Hunt & Ottoson, 1975; Hunt, 1990; De-Doncker *et al.*, 2003). 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. The CV of each afferent fiber 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.

Data analysis

The CV data of muscle spindle afferents were pooled in each age group. All means are given along with the standard deviation (S.D.). The effects of aging 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 $P<0.05$.

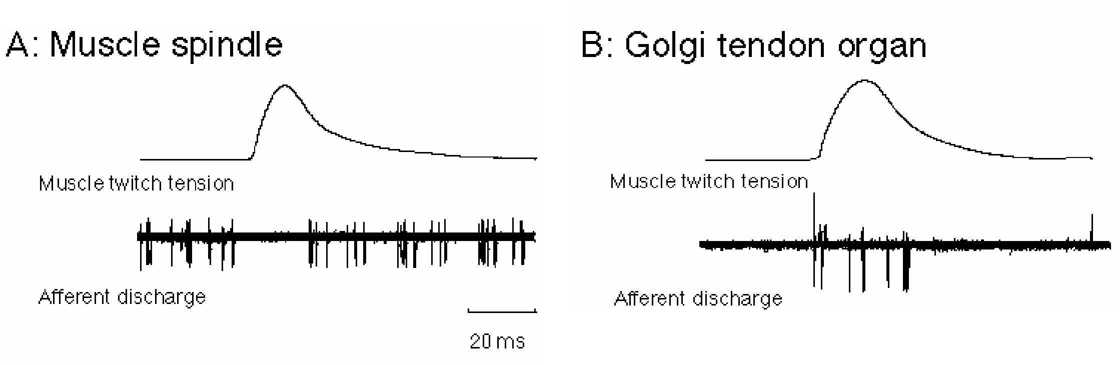

Figure 3.1. Identification of muscle spindle (A) and Golgi tendon organ (B).

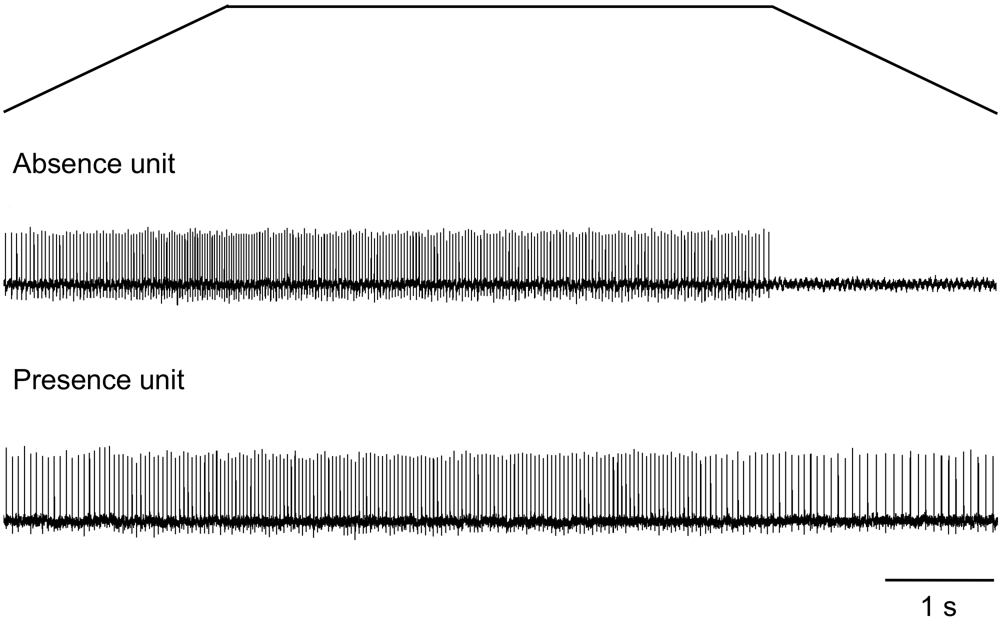

Figure 3.2. Classification of afferents into Absence and Presence units. *upper row*: displacement of stretch, *middle row*: discharge of Absence unit, *lower row*: discharge of Presence unit.

Results and Discussion

In all, 197 spindle afferents were analyzed: 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. Figure 3.3 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, 33-50 m/s, and 21-50 m/s. Those of Absence units in young, middle-aged and old rats were 52-74 m/s, 53-66 m/s, and 35-50 m/s, respectively. The CV distribution showed two peaks in young rats, although they overlapped slightly; also, a dividing point at 50 m/s 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.

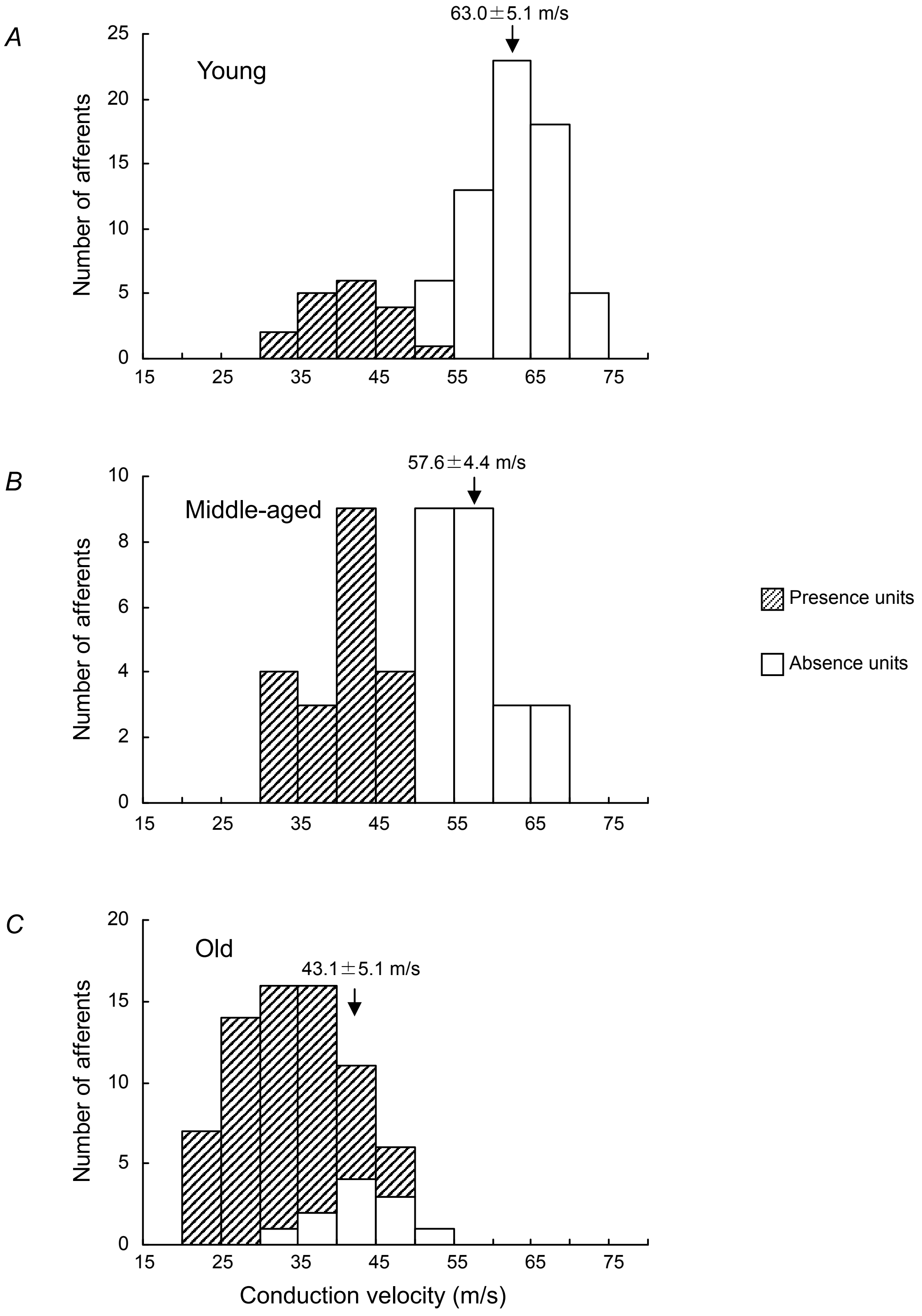

Figure 3.3. Distribution histogram of conduction velocity of muscle spindle afferents in young (A), middle-aged (B) and old (C) rats.

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), although primary and secondary CVs show a slight overlap. Similarly, the present experiments classified muscle spindle afferents into Absence and Presence units as two peaks in young and middle-aged rats. Therefore, the results obtained in this study suggest that our Absence units corresponded to primary endings and our Presence units to secondary endings in young and middle-aged rats. On the other hand, the CVs in old rats were lower for both Absence (31.7%) and Presence (20.9%) units than those of young or middle-aged rats; they also showed a unimodal distribution. Age-related changes of sensory and motor nerve CV in human have been described as linear (Lafratta & Canestrari, 1966; Dorfman & Bosley, 1979) or nonlinear (Trojaborg, 1976; Bouche *et al*., 1993). Chase *et al*. (1992), using an electrophysiological and morphological study of cat masseter nerves, described that an age-dependent decrease in CV was related to decrease axon diameter and thinning of the myelin sheath. Furthermore, it has been reported that neurophysiological responses that are mediated by large diameter nerve fibers deteriorate with age, whereas those that are dependent on small fibers are preserved (Verdu *et al*., 1996). A previous experiments (Sugiura & Kanda, 2004) revealed that the decrease in CV of motoneurons with aging 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 consequently small. There is also some evidence of loss of primary sensory neurons and overall extent perikaryal projections in spinal ganglion neurons with age (Bergman & Ulfhake, 1998; Pannese *et al*., 1998). Bergman & Ulfhake found that the dorsal root ganglion neurons (DRG) of aged rats have a smaller mean cross-sectional area at the fifth cervical (C5) and fourth lumbar (L4) levels. Neurofilament marker RT97 DRG neurons of the aged rats have significantly smaller cross-sectional areas than those of the young adult rats, indicating a selective cell body atrophy among myelinated primary afferents during aging.

Experiment 2: Dynamic response to ramp-and-hold stretch

Methods

Animals, surgical procedure, and experimental protocol

Animals and surgical procedure were the same as in Experiment 1. Following Experiment1, single afferent discharges of the muscle spindles in the gastrocnemius muscle were recorded during ramp-and-hold stretch. The muscle was stretched by 2.0 mm at 2, 4, 10, and 20 mm/s, followed by a 5 s plateau and a 1 s release phase. The initial muscle length was set at a length that generates muscle tension of about 50 g. Each series of stretches was repeated six times every 15 s. Muscle spindles' afferent spikes were recorded using a digital tape recorder (Instrumentation Cassette Recorder PC-108; Sony Corp., Japan) for storage.

Data analysis

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 are illustrated in Fig. 3.4. Several parameters were measured: the peak frequency (PF) that was the maximum frequency at the end of the dynamic stretch, the dynamic index (DI) that was the difference between the PF and the frequency at 0.5 s after completion of the stretch (Crowe & Matthews, 1964). 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 data of muscle spindle afferents were pooled in each age group. All means are given along with the standard deviation (S.D.). The statistical analysis was identical to that used in Experiment 1.

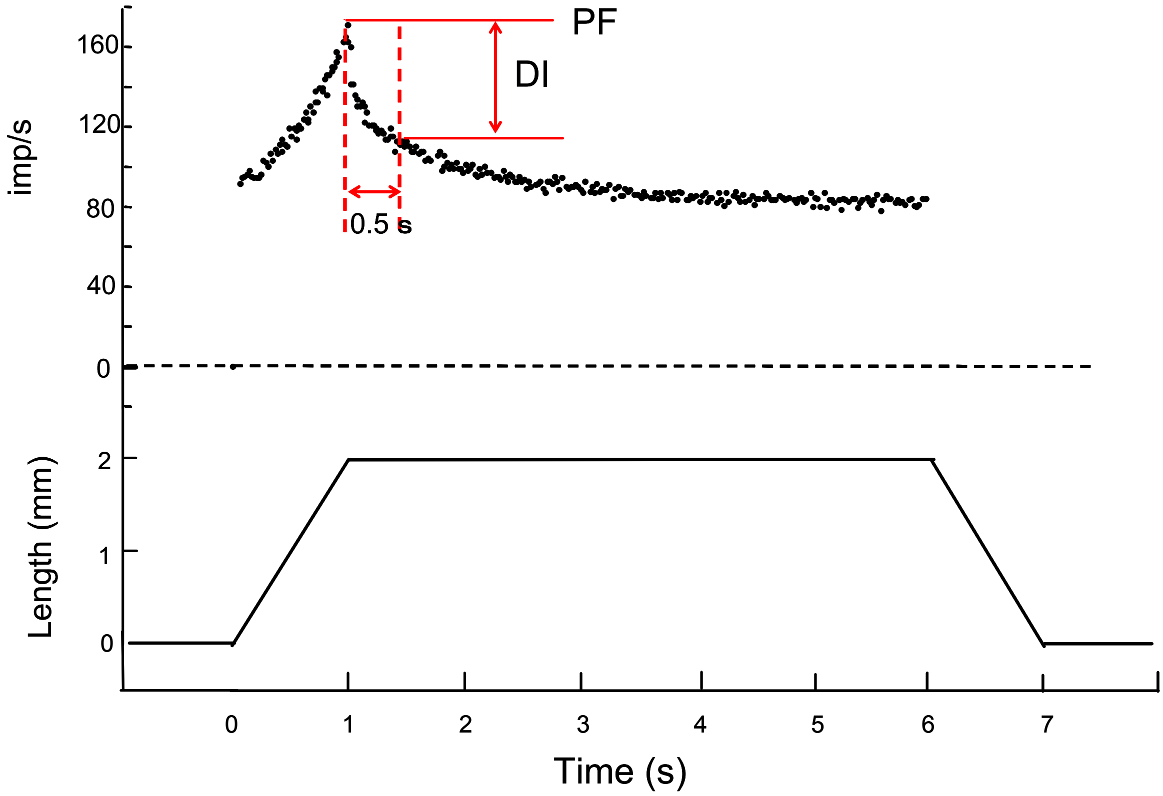

Figure 3.4. Typical response to ramp-and –hold stretch. *upper row*: instantaneous discharge frequency of muscle spindle afferents, *lower row*: displacement of stretch, *DI*: dynamic index, *PF*: peak frequency.

Results and Discussion

The 197 spindle afferents which studied in Experiment 1 were analyzed (Presence units: 18 afferents in young rats, 20 afferents in middle-aged rats, and 60 afferents in old rats; Absence units: 64 afferents in young rats, 24 afferents in middle-aged rats, and 11 afferents in old rats). Figure 3.5 shows the example of DI at 20 mm/s in young and old rats. The DI of old rats obviously decreased than those of young rats. The decrease of DI in old rats was mainly attributable to the low peak frequency (PF). The discharge 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 ± 71.1 imp/s at 20 mm/s), although they rose slowly in old rats (PF, 138.6 ± 45.6 imp/s). The discharge frequency of muscle spindle afferents at 0.5 s after completion of ramp stretch was similar for young (92.3 ± 32.8 imp/s) and old rats (91.1 ± 29.6 imp/s).

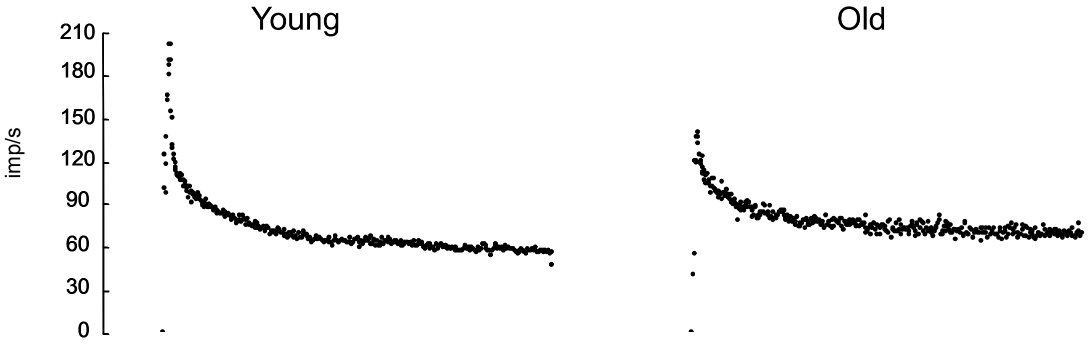

Figure 3.5. Examples of instantaneous discharge of muscle spindle afferents to ramp stretch. *left:*a young rat, *right*: an old rat.

The DI increased concomitant with the increased stretch velocity in all age group. A linear relationship was found between these two parameters when they were logarithmically converted (Fig. 3.6A). The slopes of the regression lines (exponent coefficients), which indicated dynamic sensitivity, for Absence units of muscle spindles in old rats (0.45 ± 0.07 imp/s per mm/s) were significantly smaller than those for muscle spindles in young and middle-aged rats (0.53 ± 0.05 and 0.51 ± 0.05 imp/s per mm/s, respectively), but those of Presence units did not differ (Figs. 3.6B-3.6D).

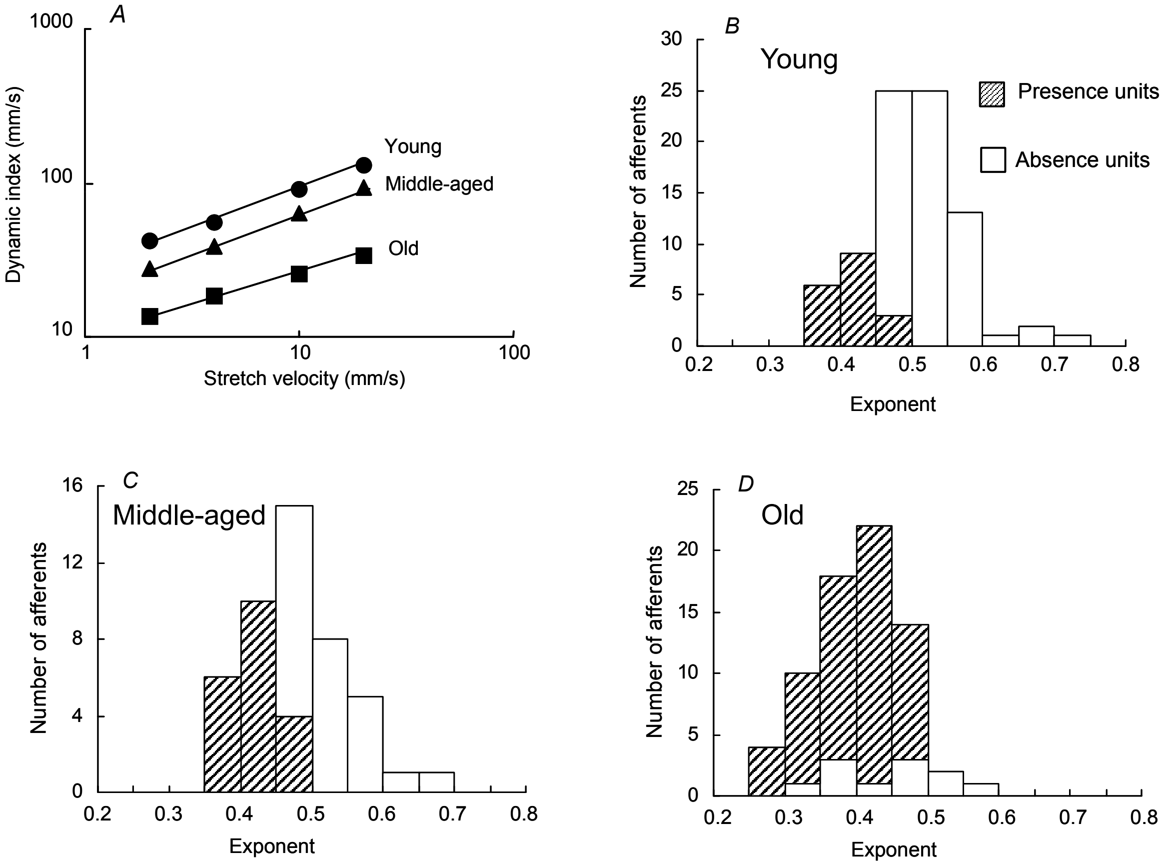

Figure 3.6. The representative relationship between DI (log) and stretch velocities (log), indicating the dynamic sensitivity (A), and the distribution of the slopes of the regression lines (exponent coefficients) for Absence and Presence units of muscle spindles in young (B), middle-age (C), and old (D) rats.

The exponent coefficients of Presence and Absence units in old rats were overlapped over a wider range (0.3-0.5 imp/s per mm/s) than for young and middle-aged rats (0.45-0.5 imp/s per mm/s). Figure 3.7 shows DI at 20 mm/s plotted against CV for the three age groups. The Presence units of young and middle-aged rats were distributed around lower DI (45.3 ± 18.9 and 46.9 ± 17.3 imp/s, respectively) and slower CV (42.5 ± 5.3 and 40.5 ± 4.6 m/s, respectively). The Absence units of young and middle-aged rats were distributed around higher DI (125.8 ± 39.5 and 128.5 ± 30.6 imp/s, respectively) and faster CV (63.0 ± 5.1 and 57.6 ± 4.4 m/s, respectively). In contrast, as shown in Fig. 3.7C, both Presence and Absence units in old rats were distributed around lower DI (39.4 ± 13.8 and 85.3 ± 53.7 imp/s, respectively) and slower CV (33.6 ± 6.3 and 43.1 ± 5.3 m/s, respectively). 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 present experiments showed that the peak frequency in old rats 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

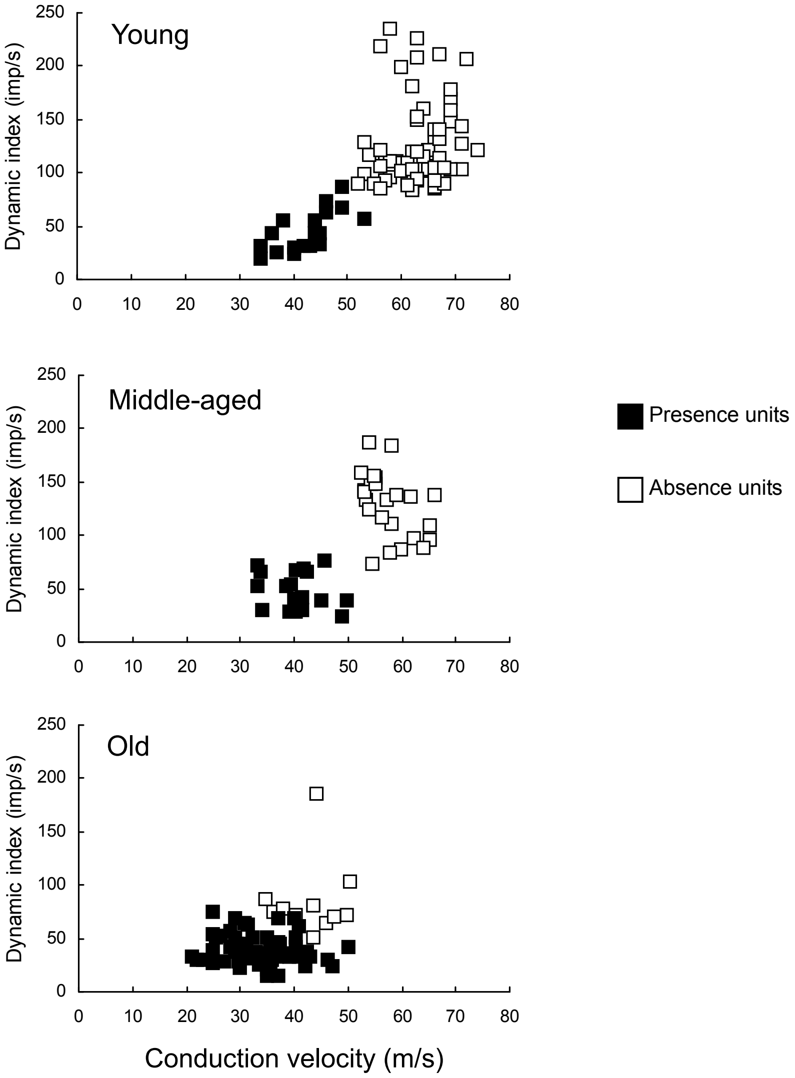

Figure 3.7. Relationship between dynamic index at 20 mm/s and conduction velocity for young (A) rats, middle-aged (B), and old (C) rats.

properties of intrafusal muscle fibers (Boyd & Smith, 1984). Indeed, Fischer & Schafer (2000) investigated effects of varying the external Ca^{2+} concentration in the isolated cat muscle spindle. They found a decrease in both the impulse activity and the sensitivity to stretch in a high Ca^{2+} solution and inverse effects in a low Ca^{2+} solution.

They discussed that the Ca^{2+} ions act directly on ion channels of the sensory membrane of muscle spindle endings. Regarding mechanical properties of intrafusal fibers, Matthews (1964) suggested that the pattern of sensory discharge might occur as a result of visco-elastic properties of the spindle receptor. That concept has been tested by Toyama (1966) and Crowe (1970) using theoretical models. Direct observation of spindles revealed that the bag fibers behave in a visco-elastic manner, while the chain fibers appear to be almost elastic (Boyd, 1966; Gladden, 1972; Cooper & Gladden, 1974; Boyd & Ward, 1975). A previous study reported that this different arrangement of elastic fibers round intrafusal fibers might be contribute greatly to the production of the dynamic response of the primary (Gladden, 1972). The primary sensory ending is wrapped around both the viscous bag and the elastic chain fibers, whereas the secondary sensory ending lies principally around the elastic chain fibers (Banks *et al.*, 1982; Boyd, 1985). Therefore age-related changes in the ionic and mechanical properties of intrafusal fibers might engender decrease of the dynamic response of the primary endings.

Experiment 3: Effects of Succinylcholine

Methods

Animals and *surgical procedure*

Experiment was performed in 34 rats which used Experiments 1 (16 rats from young group, 10 rats from middle-aged group, and 18 rats from old group). Surgical procedure was the same as in Experiment 1. Before giving the muscle relaxant (SCh), it was confirmed that anesthesia was sufficiently deep to suppress the flexion reflex to paw pinch.

Experimental protocol

Following Experiment 2, the control response to a ramp-and-hold stretch was measured (2 mm at 10 mm/s). Then the effects of SCh on the stretch responses were tested by i.v. injection of SCh (200 μg/kg) during ramp-and-hold stretches. A minimum time of 30 min was allowed between does of SCh, which was found by trial to be sufficient tome to allowed complete recovery.

Data analysis

The effect of SCh was estimated by comparing DI before and after i.v. injection of SCh. Increase in DI caused by SCh are symbolized by the prefix Δ, thus ΔDI is

SCh DI－control DI. The data of muscle spindle afferents were pooled in each age group. All means are given along with the standard deviation (S.D.).

Results and Discussion

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. Most units indicated an increased discharge at 30 s after SCh and reached a peak value at 60-120 s, with full recovery at 1200 s. The representative effects of SCh in young rats are illustrated in Fig.3.8A. Absence units showed slightly slowing discharge during the release phase and a large increment of DI, whereas Presence units showed a small increment of DI. The mean ΔDI of Absence and Presence units in young rats were 18.9 ± 14.1 and 2.2 ± 3.9 imp/s, respectively, and those of in middle-aged were 30.9 ± 21.3 and 3.4 ± 3.7 imp/s, respectively. In contrast, as shown in Fig. 3.8B, the DI of Absence units in old rats showed a small increment cased by SCh. The mean ΔDI of Absence and Presence units were 4.9 ± 9.1 and 1.2 ± 1.5 imp/s, respectively. Consequently, the injection of SCh helped clarify the classification of spindle afferents in young and middle-aged rats (Figs. 3.9A and 3.9B), but it did not allow two group endings in old rats to be clearly distinguished (Fig. 3.9C).

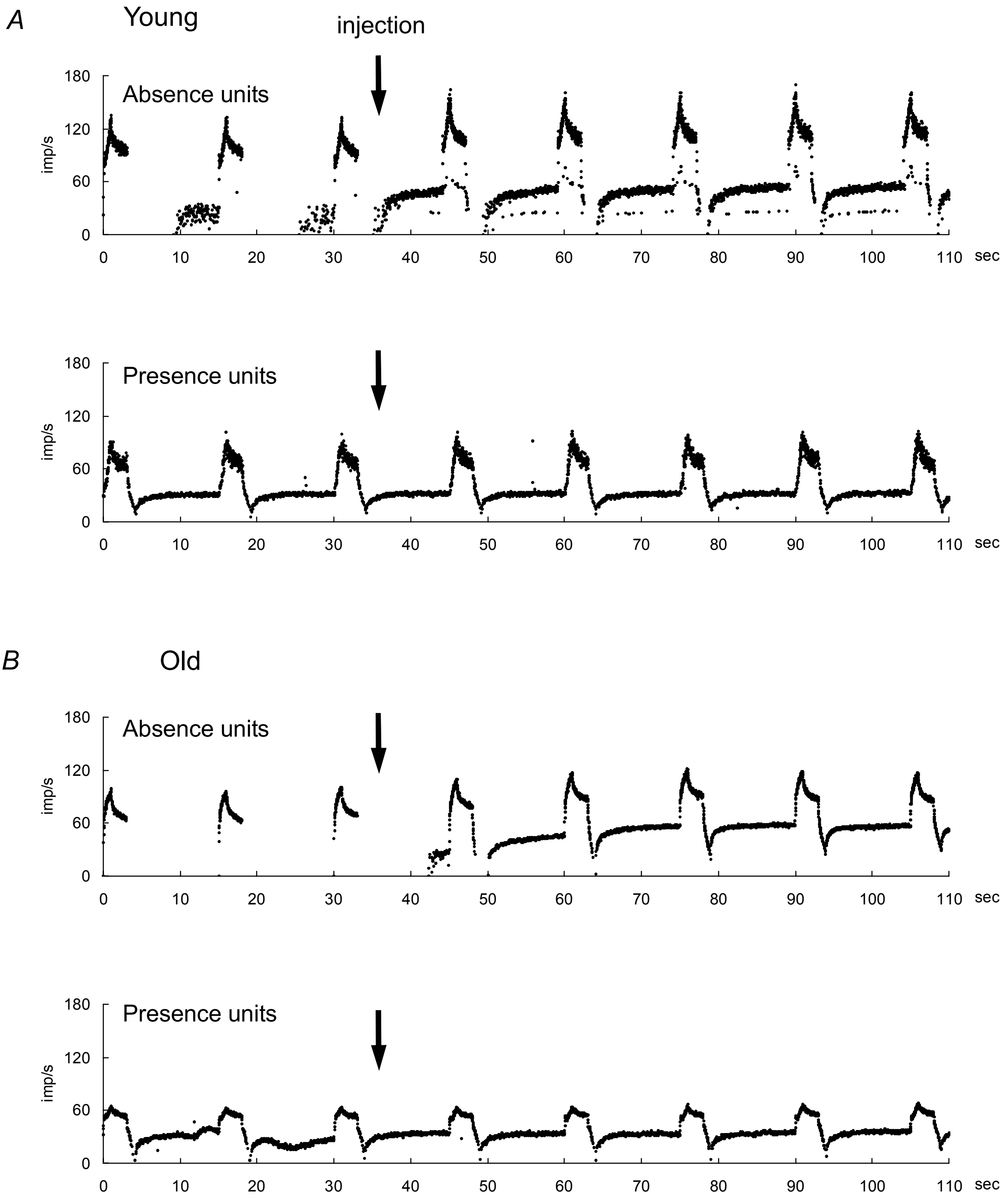

Figure 3.8. Representive response to ramp-and-hold stretch before and after SCh injection for Absence and Presence units in young (A) and old (B) rats. *arrows*: injection of i.v. SCh (200 µg/kg).

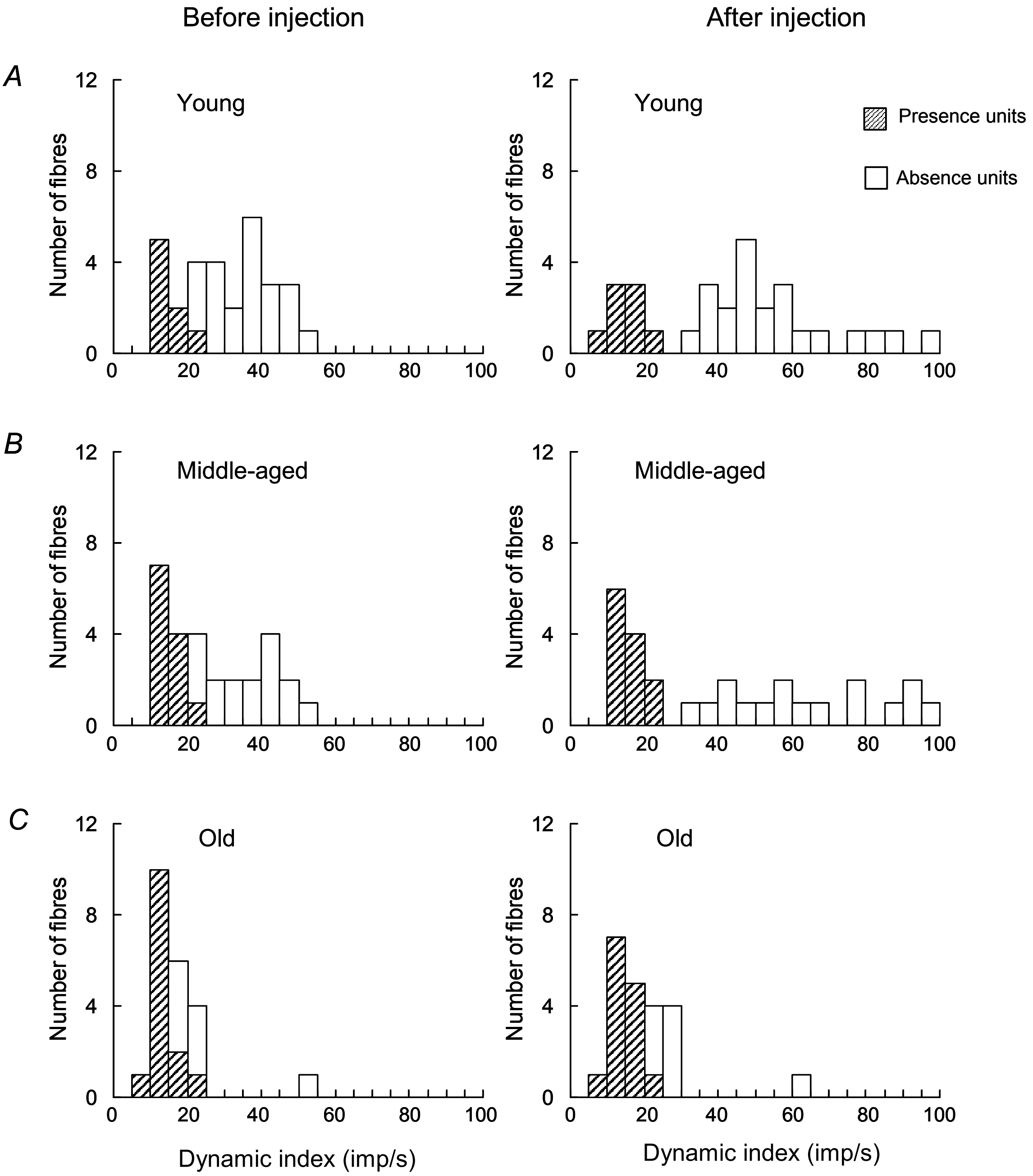

Figure 3.9. Distribution histograms of the values of DI of Absence and Presence units before and after SCh injection in young (*A*), midlle-aged (*B*), and old (*C*) rats.

Rack & Westbury (1966) introduced the use of SCh for classification of muscle spindle afferents and reported the effect of SCh to be a large increase in the dynamic response of primary endings more than secondary endings to muscle stretch, similar to the effect of dynamic fusimotor stimulation. The excitation by SCh of the spindle primary endings has been known to cause intrafusal contraction of the two nuclear bag fibers and not of the chain fibers (Gladden, 1976). Duita (1980) suggested that the excitation of secondary endings, which exist mainly on the chain fibers, is probably the result of depolarization of the afferents nerve terminals. Therefore, our results, which show lack of increasing DI by SCh of aged rats, suggest that the mechanical properties of the nuclear bag fibers might be changed with age. To our knowledge, however, no study to data has examined age-related changes in the mechanical properties of intrafusal muscle fibers.

Another reason for the decline of dynamic response in aged rats might be changes in the distribution of primary and secondary terminations on the different intrafusal fibers. Taylor and collaborators (1992) reported that the classification of muscle spindle afferents can be enhanced by a population statistical approach and proposed that muscle spindle afferents from the cat jaw closer muscles are divisible into four subgroups (b_1c, b_1b_2c, b_2c, and c) on the basis of their response to SCh. This technique yields information about the distribution of the intrafusal fibers and of the afferent terminals lying on them. Actually, we also tested a similar parameter made 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 aged rats because of the few samples that were used. As described with the results of morphological study, there might be no changes in distribution of terminations on the different intrafusal fibers for either primary or secondary endings with aging. More details of age-related changes in mechanical properties of intrafusal fibers and distribution of terminations on different intrafusal might be required in future studies.

Experiment 4: Response to sinusoidal stretch

Methods

Animals, surgical procedure, and experimental protocol

Experiment was performed in 9 rats which used Experiments 1 (2 rats from young group, 2 rats from middle-aged group, and 9 rats from old group). Surgical procedure was the same as in Experiment 1. Following Experiment 3, the muscle afferent responses to sinusoidal stretch were recorded. The muscle was stretched using a sinusoidal wave (amplitude, 0.02-2.0 mm; frequency, 0.5 Hz).

Data analysis

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. 3.10A). We estimated the linear range within which the response amplitude of afferents to the amplitude of stretch is highly linear. Figure 3.10B 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).

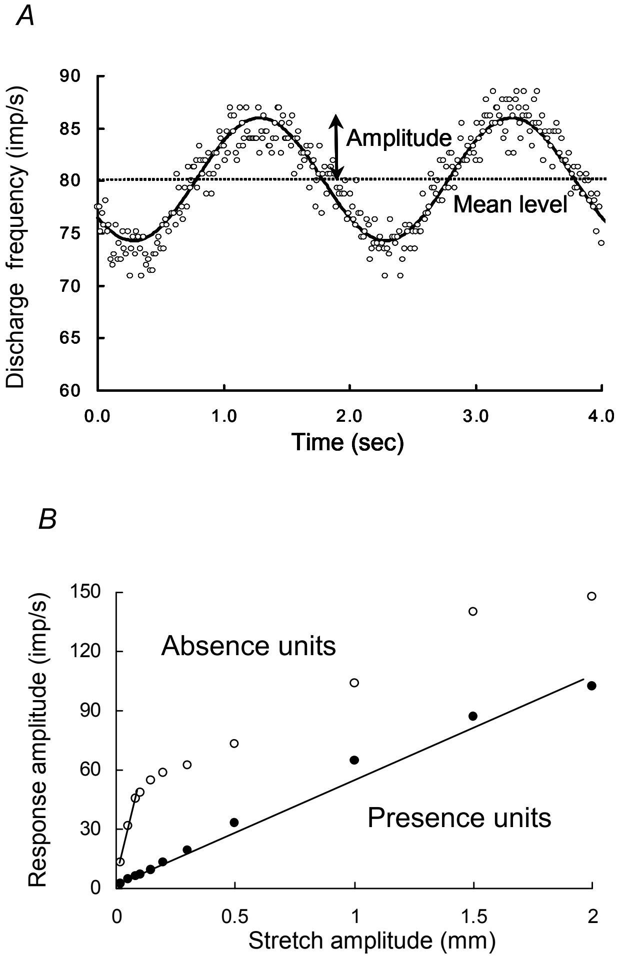

Figure 3.10. Measurement of the response of muscle spindle afferents to sinusoidal stretch. *A*: instantaneous discharge of muscle spindle afferents and the fitted sine curve, *B*: respective relations between the amplitudes of stretch and the response of Presence and Absence units.

Results and Discussion

Of the 197 spindle afferents studied previously under ramp-and-hold stretch, 24 were analyzed using sinusoidal stretch (5 afferents from young rats, 5 afferents from middle-aged rats, and 14 afferents from old rats). The typical relationships between response to ramp-and-hold stretch and response to sinusoidal stretch are illustrated in Fig.3.11. The Absence unit under ramp-and-hold stretch was discontinued under sinusoidal stretch and the Presence unit under ramp-and-hold stretch was continued under sinusoidal stretch.

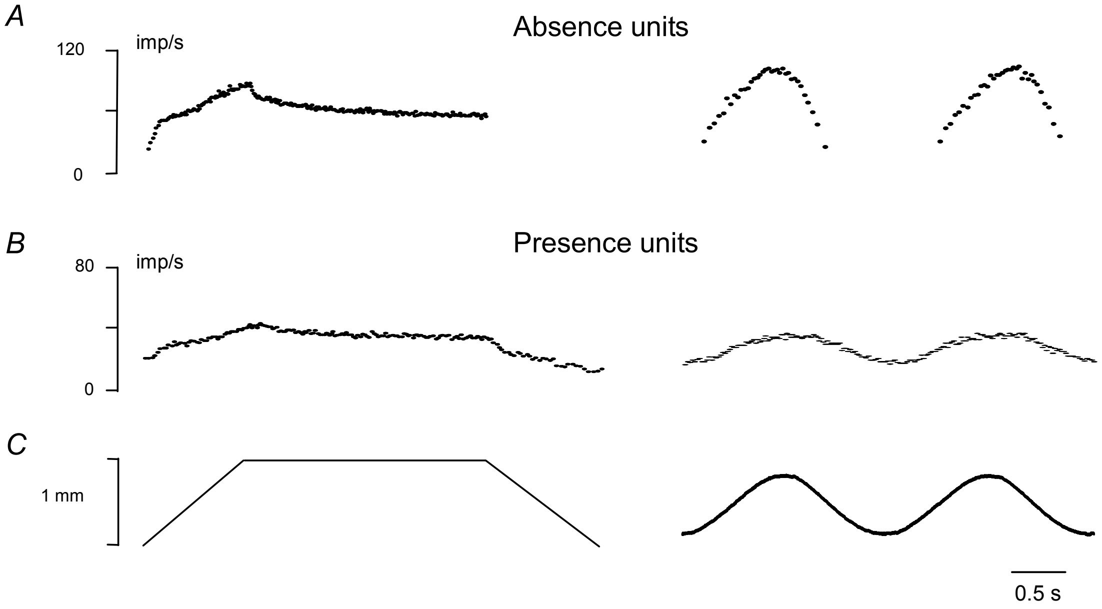

Figure 3.11. Relationships between response to ramp-and-hold stretch and response to sinusoidal stretch. *A:* Absence unit under ramp-and-hold stretch, *B*: Presence unit under ramp-and-hold stretch, and *C*: Muscle displacement.

The responses of all 24 units were plotted against the amplitude of stretch (Fig 3.12.) 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). 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) and suggested that our Absence units corresponded to primary endings and that our Presence units to secondary endings. 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.

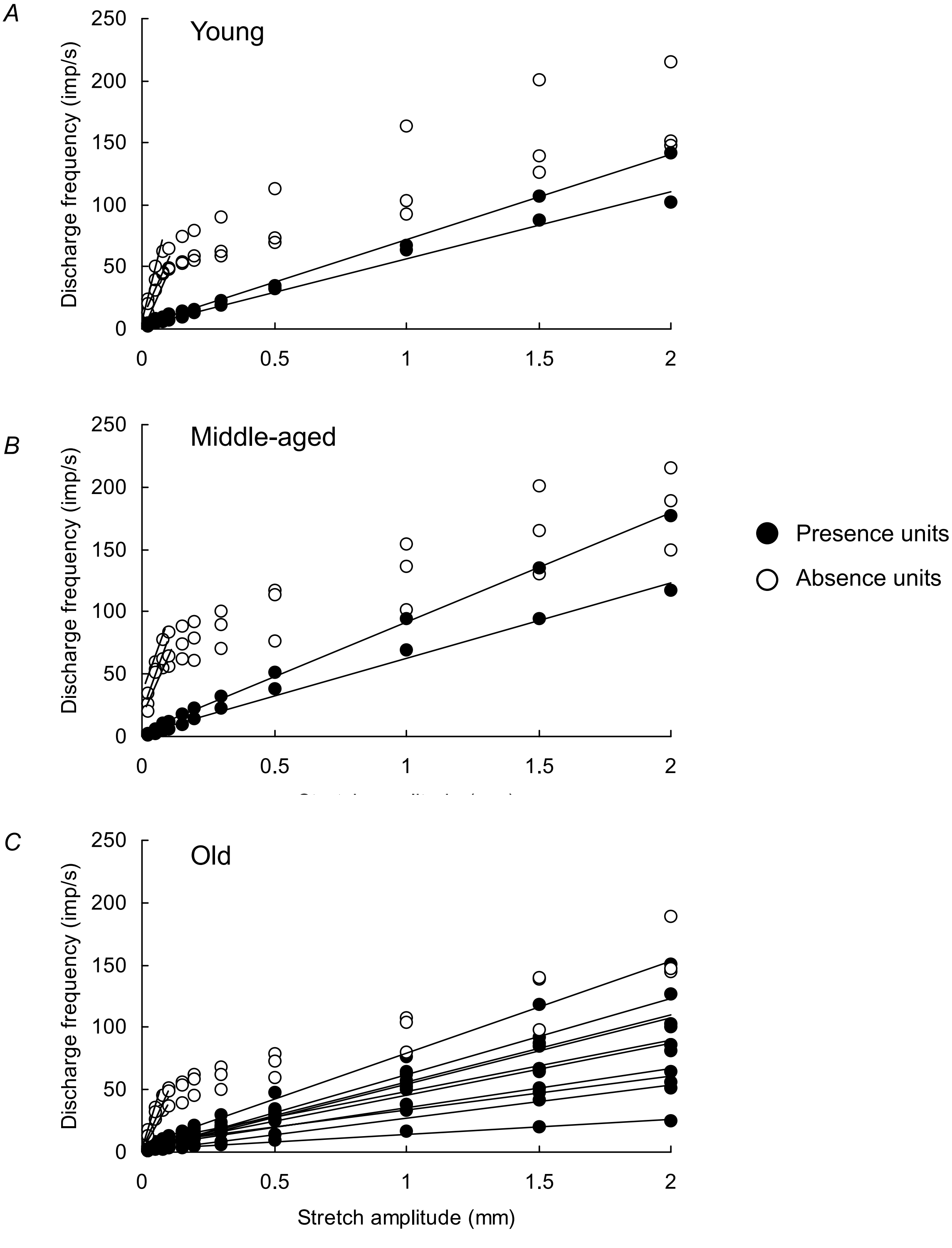

Figure 3.12. Relationship between the amplitude of stretch and the response in young (*A*), middle-aged (*B*), and old (*C*) rats.

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.

CHAPTER IV

MORPHOLOGICAL STUDY

Our physiological findings suggested two possibilities: preferred dropout of the primary endings 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. The aim of morphological study is the investigation of age-related morphological changes in sensory endings by teased silver-impregnated muscle spindles. If primary endings disappear in aged teased-preparation, the morphological results would support the former possibility. Alternatively, if primary endings exist in aged teased-preparation, it would support the latter possibility. Through addition of morphological quantitative studies of spindle afferents, we re-examined age-related changes of muscle spindles in old rats.

Methods

Animals

Experiment was performed in 10 rats which used electrophysiological experiment (3 rats from young group, 3 rats from middle-aged group, and 4 rats from old group).

Experimental protocol

After electrophysiological experimentation, the medial gastrocnemius muscle was dissected and immersed immediately in freshly prepared fixing solution. Then we processed according to Barker & Ip's (1963) technique, silver impregnation staining method, in order to carry out morphological observation. They indicated that the method has the following advantages: i) it can be applied with equal success to both small and large limb muscles, ii) the outer layers of tissue are not overstained; the quality of impregnation is remarkably uniform throughout, iii) both nervous and non-nervous elements are presented with a degree of clarity, detail, and completeness not seen in either gold chloride or methylene blue preparation. The precise details are as follows: i) fix muscle for 4-6 days in freshly prepared chloral hydrate, 1 g; 95% alcohol, 45 ml.; distilled water, 50 ml.; conc. nitric acid, 1 ml., ii) wash for 24 hr in running tap water, iii) place for 24-48 hr in 95% alcohol, 25 ml.; ammonia, 1 drop, iv) incubate for 5 days in 1.5% silver nitrate at 37℃, v) reduce for 2 days in freshly prepared hydroquinone, 2 g; 25% formic acid, 100ml., vi) rinse in distilled water and store in glycerin before teasing. 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 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 fibers), number of bands for each intrafusal fiber, 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 analysis

The data of morphometrics of primary and secondary endings was calculated for each muscle spindle and then the mean value for each age group was obtained. All means are given along with the standard deviation (S.D.). The effects of aging and differences between muscle spindle ending types were evaluated using the same statistical analysis as in Experiment 1.

Results and Discussion

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 from four old rats. Figure 4.1A 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 fiber in the equatorial region; terminations of secondary endings innervated in the juxta-equatorial region. The bag and chain fibers were readily identified by their markedly different diameters. We were unable to determine bag fiber types (bag_1, bag_2) with certainty, but terminals of primary afferent always supplied to at least one of the bag fibers in all age groups. Therefore, there might not be changes with aging in the distribution of terminations on the different intrafusal fibers 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 ± 4.7%; middle-aged, 76.2 ± 15.8%; old, 76.3 ± 9.0%). Some muscle spindles

had one primary ending and two secondary endings (young, 14.6 ± 4.9%; middle-aged, 9.2 ± 10.1%; old, 12.6 ± 11.6%) or no secondary ending (young, 8.8 ± 0.4%; middle-aged, 14.6 ± 5.9%; old, 11.1 ± 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 (Figs. 4.1B and 4.1C), 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. 4.2A), less spiral (i.e., flat and spiral, as shown in Fig. 4.2B), and non-spiral (i.e., not at all spiral, as shown in Fig. 4.2C). 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 ± 0.5% and 40.5 ± 9.3% for each muscle, (Table 4.1).

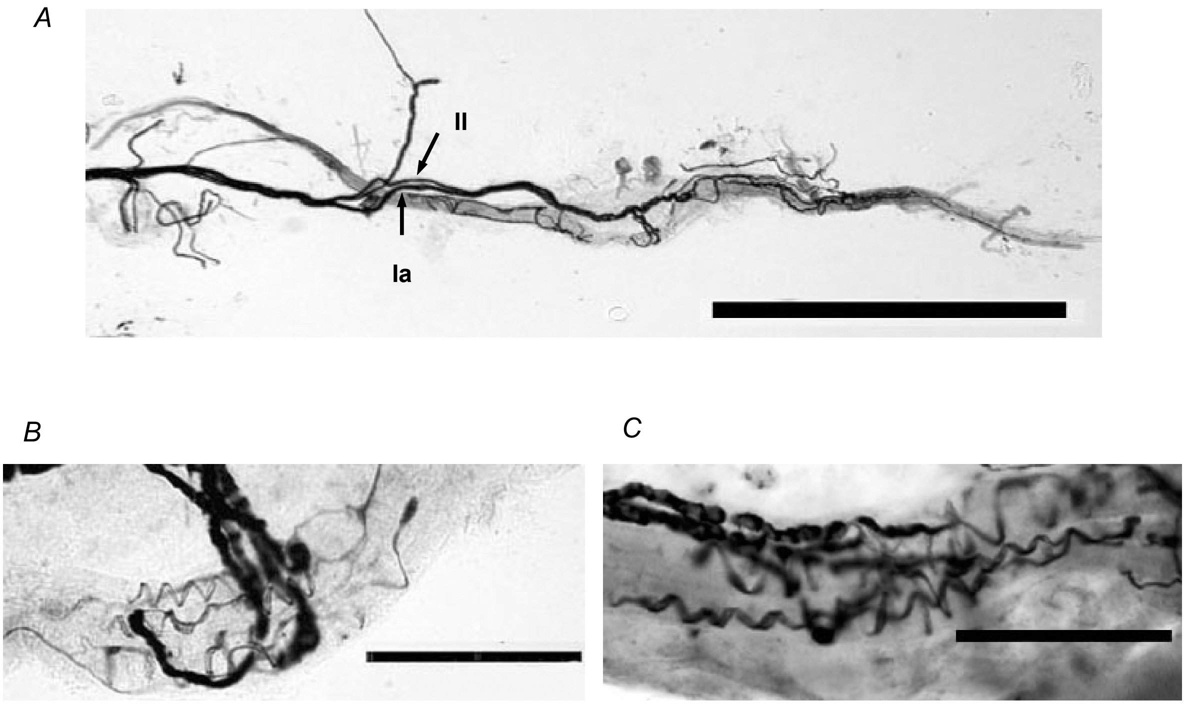

Figure 4.1. Example of teased silver-impregnated muscle spindles viewed under a light microscope. *A*: all silver-impregnated spindles isolated from the medial gastrocnemius muscles of a young rat. Arrows respectively indicate Group I and Group II. *B* and *C*: equatorial regions of muscle spindles in 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.

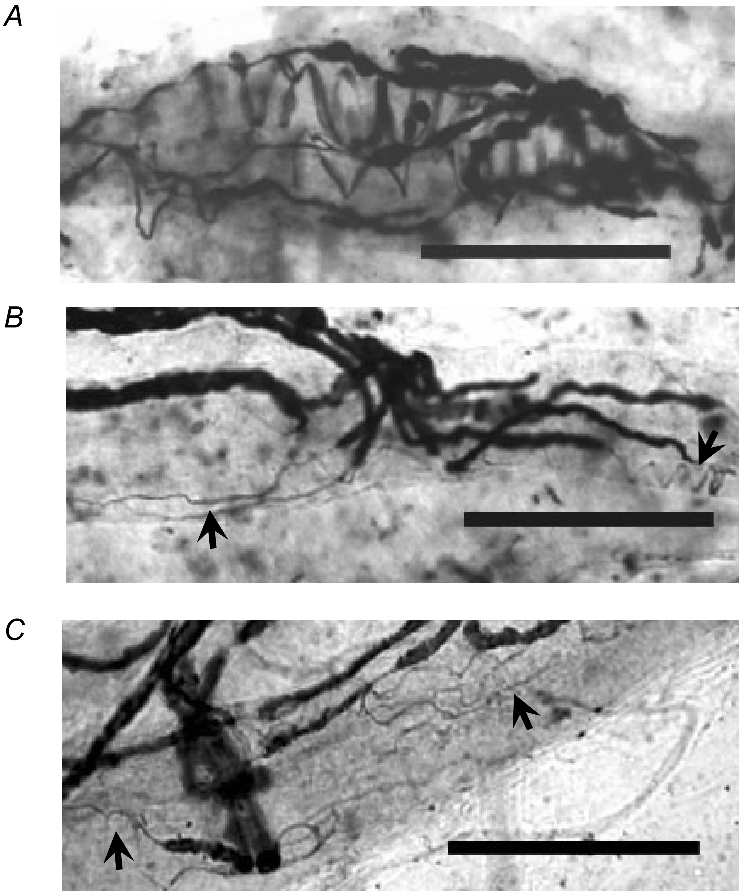

Figure 4.2. Equatorial regions of muscle spindles in an old rat. Primary endings are three muscle spindle types in old rat: as spiral (A), less spiral (B), and non-spiral (C). Arrows in B and C indicate tapered and irregularly shaped sensory terminals. See text for type definitions. Scale bars indicate 100 μm in A, B and C.

Table 4.1. Numbers of muscle spindle types from medial gastrocnemius muscles of young, middle-aged, and old rats.

Age Groups	Number of Spindles	Spiral	Less Spiral	Non-Spiral
Young #1	11	11	0	0
Young #2	12	12	0	0
Young #3	11	11	0	0
Middle-aged #1	10	10	0	0
Middle-aged #2	12	11	1	0
Middle-aged #3	13	13	0	0
Old #1	13	2	6	5
Old #2	11	2	5	4
Old #3	13	0	6	7
Old #4	15	3	7	5

The mean length of 45 primary endings (longitudinal extent of unmyelinated endings that lie along fibers) in young rats was 167.1 ± 32.8 µm. That of 34 primary endings in middle-aged rats was 165.9 ± 24.0 µm. In contrast, 81 primary endings of old rats had mean length of 126.8 ± 24.8 µm (Table 4.2). 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 fiber were significantly fewer than for those of young and middle-aged rats (Table 4.2). 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. Figure 4.3 shows that the mean diameters of both Ia and II afferents in old rats (3.5 ± 0.7 and 2.3 ± 0.7 µm, respectively) were significantly thinner than those of young (5.7 ± 0.6 and 3.2 ± 0.7 µm) and middle-aged rats (5.4 ± 0.7 µm and 3.0 ± 0.6 µm).

Table 4.2. Mean length of endings and mean number of transverse terminal bands.

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

Numbers of less spiral and non-spiral endings are shown in parentheses. *: $P<0.01$

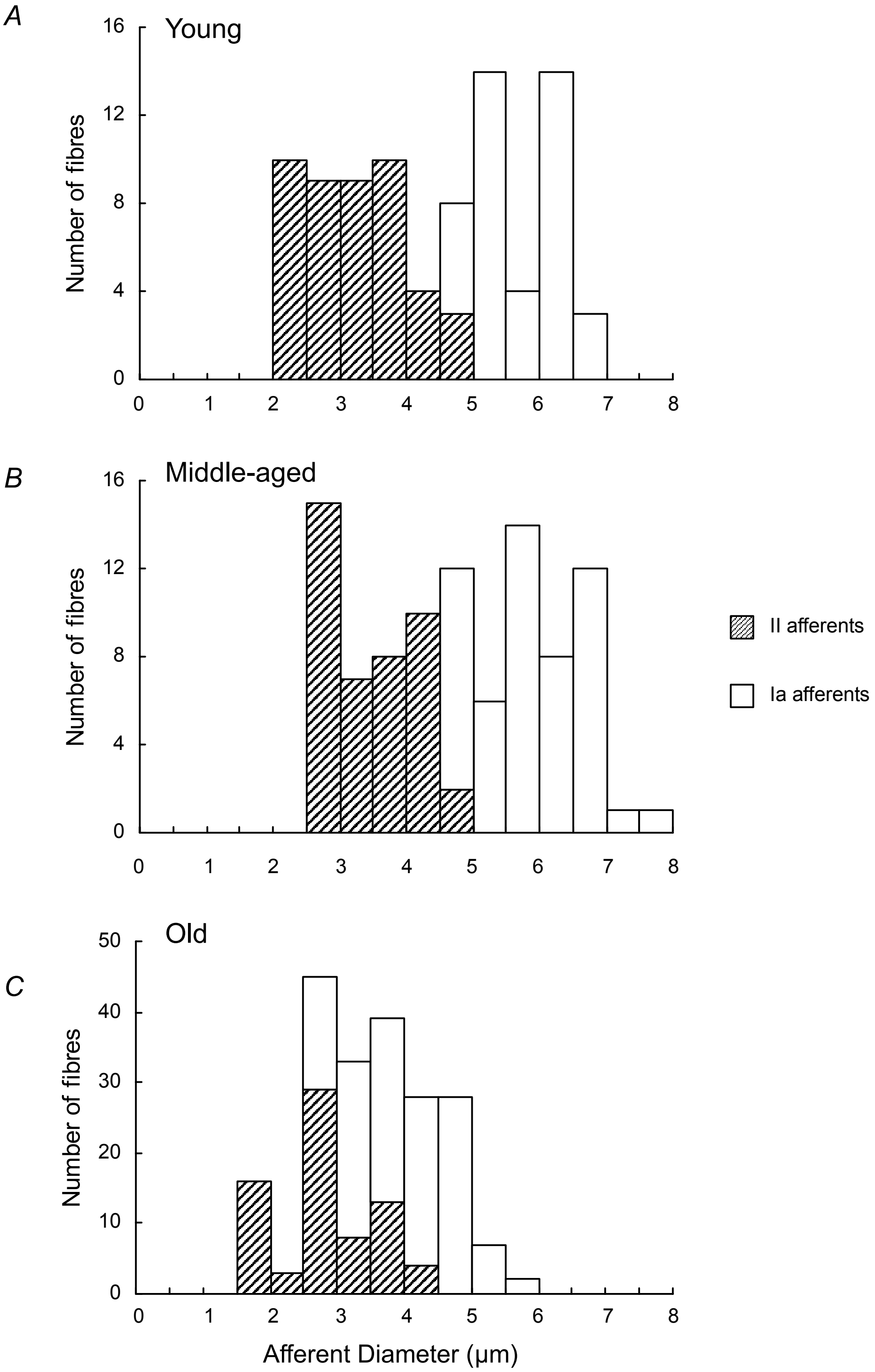

Figure 4.3. Distribution histograms of the diameters of Ia and II afferents as near muscle spindle entry. *A*: forty spindle Ia afferents and forty-five II spindle afferents in young rats. *B*: fifty-two spindle Ia afferents and forty-three II spindle afferents in middle-aged rats. *C*: one hundred twenty-eight spindle Ia afferents and seventy-three II spindle afferents in old rats.

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 inter-individual 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 fibers 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 fibers enlarge, and occasionally parts of the equatorial region of intrafusal fibers 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 fibers and their motor nerve endings (Swash & Fox, 1972, but see also Winarakwong *et al*., 2004).

CHAPTER V

GENERAL DISCUSSION

We sought to determine the age-related change in muscle spindles. In electrophysiological experiments, we first attempted to classify spindles as having primary and secondary endings in aged rats using electrophysiological criteria, but they provide no clear distinction because of the great overlap of Absence and Presence units in old rats, even after injection of SCh. Most units sampled from old rats showed properties of secondary muscle spindles seen in young rats. Consequently, we were unable to distinguish the primary endings from secondary endings in aged muscle spindles using conventional criteria. These results suggested two possibilities: preferred dropout of the primary endings occurred in aged muscle spindles; alternatively, their physiological properties changes to secondary-ending-like response. Our light microscopic observations, however, revealed that primary endings were changed into less spiral or non-spiral endings in old rats, although they still existed. These findings suggest that primary muscle spindle endings tend to become physiologically similar to secondary endings in old rats.

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 innervated extent of sensory endings on intrafusal fibers reflects a capacity for depolarization of sensory endings because the stretch of intrafusal fibers 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.

RELATED ARTICLES

1. Kim GH, Suzuki S & Kanda K (2007). Age-related physiological and morphological changes of muscle spindles in rats. *J Physiol (Lodon)* In Press.

2. Kim GH, Kanda K & Suzuki S (2002). Dynamic and static length sensitivities of muscle spindles in aged rats [Abstract]. *European Neuroscience, Abstract book of Satellite Symposium of the 3rd Forum of European Neuroscience*, *Paris, France,*30

3. Kim GH, Kojima N, Kanda K & Suzuki S (2003). Age-related changes of muscle spindles in the afferent discharge response to muscle stretch [Abstract]. P*roceedings of the Physiological Society of Japan 80th Congress, Fukuoka, Japan,* 180.

4. Kim GH, Kanda K & Suzuki S (2004). Response of the muscle spindles in aged rats to ramp-and-hold and sinusoidal stretches [Abstract]. *American Nuerosicence, Book of program session of Society for Neuroscience 34th Annual Meeting, San Diego, America,* 126-359, 24.

5. 金祉希，鈴木秀次，神田健郎.（2006）．加齢に伴う筋紡錘Ia群感覚神経の形態変化．第61回日本体力医学会，神戸，体力科学, 55, 582.

REFERENCES

Adal MN & Barker D (1962). Intramuscular diameters of afferent nerve fibres in the rectus femoris muscle of the cat. In *Symposium on Muscle Receptors*, ed. Barker D. pp. 249-256. Hong Kong: Hong Kong University Press.

Adinolfi AM, Yamuy J, Morales FR & Chase MH (1991). Segmental demyelination in peripheral nerves of old cats. *Neurobiol Aging* **12,** 175-179.

Alexander NB (1994). Postural control in older adults. *J Am Geriatr Soc* **42,** 93-108.

Arendt KW & Asmussen G (1974). Number and distribution of muscular spindles in the triceps surae muscle of the rat. *Anat Anz* **136,** 207-216.

Baloh RW, Fife TD, Zwerling L, Socotch T, Jacobson K, Bell T & Beykirch K (1994). Comparison of static and dynamic posturography in young and older normal people. *J Am Geriatr Soc* **42,** 405-412.

Balice-Gordon RJ (1997). Age-related changes in neuromuscular innervation. *Muscle Nerve Suppl* **5,** S83-87.

Banks RW & Barker D (1989). Specificities of afferents reinnervating cat muscle spindles after nerve section. *J Physiol* **408,** 345-372.

Banks RW, Barker D & Stacey MJ (1982). Form and distribution of sensory terminals in cat hindlimb muscle spindles. *Philos Trans R Soc Lond B Biol Sci* **299,** 329-364.

Barker D (1967). The innervation of mammalian skeletal muscle. In *Myotatic Kinesthetic and Vestibular Mechanisms* ed. de Reuck A.V.S & Knight J. pp. 3-15. Londond. Churchill.

Barker D & Chin NK (1960). The number and distribution of muscle-spindles in certain muscles of the cat. *J Anat* **94,** 473-486.

Barker D & Ip MC (1963). A silver method for demonstrating the innervation of mammalian muscle in teased preparations. *J Physiol*, **169,** 73-74P.

Barker D & Scott JJ (1990). Regeneration and recovery of cat muscle spindles after devascularization. *J Physiol* **424,** 27-39.

Barker D, Scott JJ & Stacey MJ (1986). Reinnervation and recovery of cat muscle receptors after long-term denervation. *Exp Neurol* **94,** 184-202.

Barker D, Stacey MJ & Adal MN (1970). Fusimotor innervation in the cat. *Phil Trans R Soc* B **258**, 315-346.

Bergman E & Ulfhake B (1998). Loss of primary sensory neurons in the very old rat: neuron number estimates using the disector method and confocal optical sectioning. *J Comp Neurol* **396,** 211-222.

Bouche P, Cattelin F, Saint-Jean O, Leger JM, Queslati S, Guez D, Moulonguet A, Brault Y, Aquino JP & Simunek P (1993). Clinical and electrophysiological study of the peripheral nervous system in the elderly. *J Neurol* **240**, 263-268.

Boyd IA (1960). The diameter and distribution of the nuclear bag and nuclear chain muscle fibers in the muscle spindles of the cat. *J Physiol* **153**, 23-24P.

Boyd IA (1962). The structure and innervation of the nuclear bag muscle fibers system and the nuclear chain muscle fiber system in mammalian muscle spindles. *Phil Trans R Soc* B **245**, 81-136.

Boyd IA (1966). The mechanical properties of mammalian intrafusal muscle fibres. *J Physiol* **187,** 10-12P.

Boyd IA (1980). The isolated mammalian muscle spindle. *Trends Neurosci* **3**, 258-265.

Boyd IA (1985). Muscle spindles and stretch reflexes. In *Scientific Basis of Clinical Neurology*. ed. Swash M & Kennard C pp. 74-96. Livingstone, London.

Boyd IA & Davey MR (1962). The groups of origin in the nerves to skeletal muscle of γ_1 and γ_2 fusimotor fibres present close to, and within mammalian muscle spindles. In *Symposium on Muscle Receptors*, ed. Barker D. pp. 191-198. Hong Kong: Hong Kong University Press.

Boyd IA, Gladden MH, McWilliam PN & Ward J (1975). ‘Static’ and ‘dynamic’ nuclear bag fiber in isolated cat muscle spindles. *J Physiol* **250**, 11-12P.

Boyd IA & Kalu KU (1979). Scaling factor relating conduction velocity and diameter for myelinated afferent nerve fibres in the cat hind limb. *J Physiol* **289**, 277-297.

Boyd IA & Smith RS (1984). The muscle spindle. In *Peripheral Neuropathy*. ed. Dyck, P. J, Thomas PK, Lombert EH & Bunge R pp. 171-202. W.B. Saunders, London.

Boyd IA & Ward J (1975). Motor control of nuclear bag and nuclear chain intrafusal fibres in isolated living muscle spindles from the cat. *J Physiol* **244,** 83-112.

Brown MC, Crowe A & Matthews PBC (1965).Observations on the fusimotor fibers of the tibialis posterior muscle of the cat. *J Physiol* **177**, 140-159.

Chase MH, Engelhardt JK, Adinolfi AM & Chirwa SS (1992). Age-dependent changes in cat masseter nerve: an electrophysiological and morphological study. *Brain Res* **586**, 279-288.

Cheney PD & Preston JB (1976). Classification and response characteristics of muscle spindle afferents in the primate. *J Neurophysiol* **39,** 1-8.

Cody FW, Lee RW & Taylor A (1972). A functional analysis of the components of the mesencephalic nucleus of the fifth nerve in the cat. *J Physiol* **226,** 249-261.

Cooper S (1961). The responses of the primary and secondary endings of muscle spindles with intact motor innervation during applied stretch. *Q J Exp Physiol* **46**, 389-398.

Cooper S & Gladden MH (1974). Elastic fibres and reticulin of mammalian muscle spindles and their functional significance. *Q J Exp Physiol Cogn Med Sci* **59,** 367-385.

Crowe A (1970). A mechanical model of muscle and its application to the intrafusal fibres of the mammalian muscle spindle. *J Biomech* **3,** 583-592.

Crowe A & Matthews PB (1964). The effects of stimulation of static and dynamic fusimotor fibres on the response to stretching of the primary endings of muscle spindles. *J Physiol* **174,** 109-131.

De-Doncker L, Picquet F, Petit J & Falempin M (2003). Characterization of spindle afferents in rat soleus muscle using ramp-and-hold and sinusoidal stretches. *J Neurophysiol* **89,** 442-449.

Desaki J (2001). Scanning electron microscopic study of muscle spindles in the tenuissimus muscle of the Chinese hamster: with reference to the outer capsule, inner capsule and fusimotor endings. *J Electron Microsc* **50**, 65-72.

Doherty TJ, Vandervoort AA, Taylor AW & Brown WF (1993). Effects of motor unit losses on strength in older men and women. *J Appl Physiol* **74,** 868-874.

Dorfman LJ & Bosley TM (1979). Age-related changes in peripheral and central nerve conduction in man. *Neurology* **29,** 38-44.

Dutia MB (1980). Activation of cat muscle spindle primary, secondary and intermediate sensory endings by suxamethonium. *J Physiol* **304,** 315-330.

Einsiedel LJ & Luff AR (1992). Effect of partial denervation on motor units in the ageing rat medial gastrocnemius. *J Neurol Sci* **112,** 178-184.

Fischer M & Schafer SS (2000). Effects of calcium on the discharge pattern of primary and secondary endings of isolated cat muscle spindles recorded under a ramp-and-hold stretch. *Brain Res* **875,** 78-88.

Gladden MH (1972). Elastic fibres in muscle spindles of the cat. *J Physiol* **227**, 45-46P.

Gladden MH (1976). Structural features relative to the function of intrafusal muscle fibres in the cat. *Prog Brain Res* **44,** 51-59.

Gregory JE & Proske U (1987). Responses of muscle receptors in the kitten to succinyl choline. *Exp Brain Res* **66,** 167-174.

Hasan Z & Houk JC (1975). Transition in sensitivity of spindle receptors that occurs when muscle is stretched more than a fraction of a millimeter. *J Neurophysiol* **38,** 673-689.

Hashizume K*,* Kanda K & Burke RE (1988). Medial gastrocnemius motor nucleus in the rat: age-related changes in the number and size of motoneurons. *J Comp Neurol* **269,** 425-430.

Hausdorff JM, Mitchell SL, Firtion R, Peng CK, Cudkowicz ME, Wei JY & Goldberger AL (1997). Altered fractal dynamics of gait: reduced stride-interval correlations with aging and Huntington's disease. *J Appl Physiol* **82,** 262-269.

Hulliger M, Matthews PB & Noth J (1977). Static and dynamic fusimotor action on the response of Ia fibres to low frequency sinusoidal stretching of widely ranging amplitude. *J Physiol* **267,** 811-838.

Hunt CC (1954). Relation of function to diameter in afferent fibres of muscle nerve. *J Gen Physiol* **38,** 117-131.

Hunt CC (1990). Mammalian muscle spindle: peripheral mechanisms. *Physiol Rev* **70,** 643-663.

Hunt CC & Ottoson D (1975). Impulse activity and receptor potential of primary and secondary endings of isolated mammalian muscle spindles. *J Physiol* **252,** 259-281.

Hunt CC, Wilkinson RS & Fukami Y (1978). Ionic basis of the receptor potential in primary endings of mammalian muscle spindles. *J Gen Physiol* **71,** 683-698.

Hurley MV, Rees J & Newham DJ (1998). Quadriceps function, proprioceptive acuity and functional performance in healthy young, middle-aged and elderly subjects. *Age Ageing* **27,** 55-62.

Hyde D & Scott JJ (1983). Responses of cat peroneus brevis muscle spindle afferents during recovery from nerve-crush injury. *J Neurophysiol* **50,** 344-357.

Inoue H, Morimoto T & Kawamura Y (1981). Response characteristics an classification of muscle spindles of the masseter muscle in the cat. *Exp Neurol* **74,** 548-560.

Kakuda N (2000). Response of human muscle spindle afferents to sinusoidal stretching with a wide range of amplitudes. *J Physiol* **527 Pt 2,** 397-404.

Kanda K & Hashizume K (1989). Changes in properties of the medial gastrocnemius motor units in aging rats. *J Neurophysiol* **61,** 737-746.

Kanda K & Hashizume K (1992). Factors causing difference in force output among motor units in the rat medial gastrocnemius muscle. *J Physiol* **448,** 677-695.

Kishimoto H, Bae YC, Yoshida A, Moritani M, Takemura M, Nakagawa S, Nagase Y, Wada T, Sessle BJ & Shigenaga Y (1998). Central distribution of synaptic contacts of primary and secondary jaw muscle spindle afferents in the trigeminal motor nucleus of the cat. *J Comp Neurol* **391,** 50-63.

Knox CA, Kokmen E & Dyck PJ (1989). Morphometric alteration of rat myelinated fibers with aging. *J Neuropathol Exp Neurol* **48,** 119-139.

Kolliker A (1862). On the terminations of nerves in muscles, as observed in the frog: and on the disposition of the nerves in the frog's heart. *Proc R Soc Lon* **12,** 65-84.

Kucera J, Dorovini-Zis K, Engel WK (1978). Histochemistry of rat intrafusal muscle fibers and their motor innervation. *J Histochem Cytochem* **26**, 973-988.

Kuhne W (1863). Uber die Endigung der Nerve in den Muskeln. *Virchows Arch Path Ant Physiol* **27,** 508-523.

Lafratta CW & Canestrari R (1966). A comparison of sensory and motor nerve conduction velocities as related to age. *Arch Phys Med Rehabil* **47,** 286-290.

Lascelles RG & Thomas PK (1966). Changes due to age in internodal length in the sural nerve in man. *J Neurol Neurosurg Psychiatry* **29,** 40-44.

Lord SR & Ward JA (1994). Age-associated differences in sensori-motor function and balance in community dwelling women. *Age Ageing* **23,** 452-460.

Matthews BHC (1933). Nerve endings in mammalian muscle. *J Physiol* **78**, 1-53.

Matthews PBC (1962). The differentiation of two types of fusimotor fibre by their effects on the dynamic response of muscle spindles primary endings. *Q J Exp Physiol* **47**, 324-333.

Matthews PBC (1963). The response of de-efferented muscle spindle receptors to stretching at different velocities. *J Physiol* **168**, 660-678.

Matthews PBC (1964). Muscle spindles and their motor control. *Physiol. Rev* **44**, 219-288.

Matthews PBC (1972). Patterns of afferent response. In *Mammalian muscle receptors and their central actions*, pp. 101-139. Baltimore, Williams & Wilkins, United State America.

Matthews PBC & Stein RB (1969). The sensitivity of muscle spindle afferents to small sinusoidal changes of length. *J Physiol* **200,** 723-743.

McCloskey DI (1978). Kinesthetic sensibility. *Physiol Rev* **58,** 763-820.

Miwa T, Miwa Y & Kanda K (1995). Dynamic and static sensitivities of muscle spindle primary endings in aged rats to ramp stretch. *Neurosci Lett* **201,** 179-182.

Ochoa J & Mair WG (1969). The normal sural nerve in man. II. Changes in the axons and Schwann cells due to ageing. *Acta Neuropathol (Berl)* **13,** 217-239.

Oda K (1984). Age changes of motor innervation and acetylcholine receptor distribution on human skeletal muscle fibres. *J Neurol Sci* **66,** 327-338.

Ovalle WK & Smith RS (1972). Histochemical identification of three types of intrafusal muscle fibers in the cat and monkey based on the myosin ATPase reaction. *Can J Physiol Pharmac* **50**, 195-202.

Overstall PW, Exton-Smith AN, Imms FJ. & Johnson AL (1977). Falls in the elderly related to postural imbalance. *Br Med J* **1,** 261-264.

Pannese E, Sartori P, Martinelli C & Ledda M (1998). Age-related decrease in the overall extent of perikaryal projections in rabbit spinal ganglion neurons. *Neurosci Lett* **254,** 177-179.

Proske U, Wise AK & Gregory JE (2000). The role of muscle receptors in the detection of movements. *Prog Neurobiol* **60,** 85-96.

Quick DC & Rogers SL (1983). Stretch receptors in regenerated rat muscle. *Neuroscience* **10,** 851-859.

Rack PM & Westbury DR (1966). The effects of suxamethonium and acetylcholine on the behaviour of cat muscle spindles during dynamics stretching, and during fusimotor stimulation. *J Physiol* **186,** 698-713.

Ruffini A (1898). On the minute anatomy of the neuromuscular spindles of the cat, and on their physiological significance. *J Physiol* **23,** 190-208.

Sherrington CS (1894). On the anatomical constitution of nerves of skeletal muscles; with remarks on recurrent fibres in the ventral spinal nerve-root. *J Physiol* **17,** 211-258.

Scott JJ (1990). Classification of muscle spindle afferents in the peroneus brevis muscle of the cat. *Brain Res* **509,** 62-70.

Sorensen KL, Hollands MA & Patla E (2002). The effects of human ankle muscle vibration on posture and balance during adaptive locomotion. *Exp Brain Res* **143,** 24-34.

Sugiura M & Kanda K (2004). Progress of age-related changes in properties of motor units in the gastrocnemius muscle of rats. *J Neurophysiol* **92**, 1357-1365.

Swash M & Fox KP (1972). The effect of age on human skeletal muscle. Studies of the morphology and innervation of muscle spindles. *J Neurol Sci* **16,** 417-432.

Takamatsu M (2003). Histochemical study of the sensory endings of muscle spindles in rat longissimus muscles. J Nippon Med Sch **70**, 342-350.

Taylor A, Durbaba R & Rodgers JF (1992). The classification of afferents from muscle spindles of the jaw-closing muscles of the cat. *J Physiol* **456,** 609-628.

Toyama K (1966). An analysis of impulse discharges from the spindle receptor. *Jpn J*

Physiol **16,** 113-125.

Trojaborg W (1976). Motor and sensory conduction in the musculocutaneous nerve. *J Neurol Neurosurg Psychiatry* **39**, 890-899.

Verdu E, Buti M & Navarro X (1996). Functional changes of the peripheral nervous system with aging in the mouse. *Neurobiol Aging* **17**, 73-77.

Verdu E, Ceballos D, Vilches JJ & Navarro X (2000). Influence of aging on peripheral nerve function and regeneration. *J Peripher Nerv Syst* **5,** 191-208.

Wei JY, Kripke BR & Burgess PR (1986). Classification of muscle spindle receptors. *Brain Res* **370,** 119-126.

Welford AT (1988). Reaction time, speed of performance, and age. *Ann N Y Acad Sci* 515, 1-17.

Winarakwong L, Muramoto T, Soma K & Takano Y (2004). Age-related changes and the possible adaptability of rat jaw muscle spindles: immunohistochemical and fine structural studies. *Arch Histol Cytol* **67,** 227-240.

Yamamoto Y, Tanaka S, Tsubone H, Atoji Y & Suzuki Y (2003). Age-related changes in sensory and secretomotor nerve endings in the larynx of F344/N rat. *Arch Gerontol Geriatr* **36,** 173-183.