Int. J. Dev. Biol. 41: 1-17 (1997)

Review

Development and regeneration of muscle spindles in mammals and birds

ALFRED MAIER*

Department of Cell Biology, University of Alabama at Birmingham, Birmingham, USA

CONTENTS

***Address for reprints:** Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294, USA. FAX: 205.934-7029. e-mail: amaier@cellbio.bhs.uab.edu.

0214-6282/97/$05.00

Printed in Spain

Introduction

In this article the normal development of mammalian and avian muscle spindles and their postnatal maturation into functional receptors are reviewed, and because of its relevance to normal development, regeneration is also discussed.

Muscle spindles are mechanoreceptors that sense how far and how fast a muscle is lengthened. A muscle spindle consists of a variable number of small-diameter intrafusal muscle fibers that are innervated by sensory and motor axons. Fibers and axons are enclosed in a fusiform, multilayered outer capsule. At the midsection of the spindle the capsule bulges to create a periaxial space around the intrafusal fiber bundle. Major features of the mammalian spindle are illustrated in Figure 1. The structure of the adult vertebrate muscle spindle has been reviewed in detail by Barker (1974).

Work on development of muscle receptors predates this century. Good sources of the literature prior to 1976 are lists of articles compiled by Eldred *et al.* (1967, 1977) and papers by Zelena (1962, 1964) and Barker (1974). Development and regeneration of mammalian spindles have been reviewed during the past decade (Barker and Milburn, 1984); however, since then a steady flow of papers has produced new information which needs to be sorted. Most data on development have been collected from mammalian muscles, but some work has been published for avian spindles, permitting comparisons to be drawn between these two classes of vertebrates. Reptilian and amphibian receptors are excluded from this review because data from these groups are at best fragmentary and give no additional insight into muscle spindle development. Nevertheless, muscle spindles were first recognized in frogs where they were thought to be growth centers in postnatal muscles (Weismann, 1861; Kölliker, 1862). It took three decades and a mammalian preparation to demonstrate that they were sensory receptors (Sherrington, 1894).

Mammals

Normal development

General morphology

Time of appearance of spindles during development varies with individual muscles and species. The most frequently used animals for muscle spindle studies are mice, rats and cats, whose gestation periods are 19-20, 21 and 63 days, respectively. Assembly of intrafusal fibers is complete in mice (Kozeka and Ontell, 1981) and kittens (Milburn, 1984) at birth, and in rats by PN4 (Marchand and Eldred, 1969; Milburn, 1973; Kucera and Walro, 1990a). Initially intrafusal myotubes are as large or larger than extrafusal myotubes (Cuajunco, 1927, 1940; Maier and Eldred, 1974; Swatland, 1974), but postnatally intrafusal fibers are quickly outgrown by extrafusal fibers (Maier and Eldred, 1974; Maier, 1993a).

Abbreviations used in this paper: E, embryonic day; MHC, myosin heavy chains; PN, postnatal day

Mammalian intrafusal fibers are categorized as nuclear bag or nuclear chain fibers, according to the arrangement of nuclei at their equatorial regions (Fig. 1). The creation of nuclear bags is an early event, occurring 1-3 days after the arrival of the sensory axon (Cuajunco, 1940; Landon, 1972; Kozeka and Ontell, 1981; Kucera *et al.*, 1988a). Nuclear bags are thought to be the earliest reliable feature for identifying the young intrafusal fiber bundle in human muscles (Mavrinskaya, 1967), but at least some rat nuclear bag_2 intrafusal fibers are recognized before they can be categorized into types by equatorial nucleation (Kucera *et al.*, 1988a). In senile rats the number of extrafusal fibers in a muscle decreases, but the number of intrafusal fibers per spindle remains constant, as does the proportion of nuclear bag to nuclear chain fibers. The number of spindles remains stable throughout life (Hartung and Asmussen, 1988a,b).

The circumference of the outer spindle capsule and the volume of the periaxial space continue to grow postnatally (Maier and Eldred, 1974; Swatland, 1974). Spindle length increases until old age, but the outer capsule grows more in length than the intrafusal fiber bundle (Hartung and Asmussen, 1988a).

Afferent innervation

Mammalian muscle spindles receive two kinds of afferents: larger, early appearing primary or Ia afferents, and smaller, later forming secondary or group II afferents. With current techniques presumptive intrafusal myotubes cannot be positively identified before the arrival of primary afferents. Large and small unmyelinated axons reach the vicinity of future muscle spindles by branching from nearby nerve trunks (Cuajunco, 1927; Kucera *et al.*, 1988a). It has been claimed that sensory and motor components can be already distinguished in the nerve network of human fetuses at week 11, an early stage of spindle formation (Cuajunco, 1940).

The initial network of axons that moves towards the future muscle spindle lacks axon terminals (Zelena, 1957). Axons that make contact with the spindle anlage in the early developmental stages are unbranched and curve around or run alongside presumptive intrafusal myotubes (Kozeka and Ontell, 1981; Kucera *et al.*, 1988a). Within the developing spindle, still unmyelinated axons (Landon, 1972; Kucera *et al.*, 1988a) begin to branch at the future equatorial region (Zelena, 1964). It has been suggested that spindle afferents compete for available intrafusal myotubes because in rat soleus more afferents are seen to impinge on E18 spindles than later in development, or on adult spindles (Kucera *et al.*, 1989).

Types of intrafusal fiber in rodents receive their sensory innervation in the order in which they are formed. Nuclear bag_2 fibers are always the first to be innervated (Kucera and Walro, 1994). First order branching patterns of primary and secondary afferents (Kucera *et al.*, 1988b) and the basic morphology of the myosensory junction become established before the end of the first postnatal week (Landon, 1972; Kucera *et al.*, 1988b). When present, secondary afferents come to lie to the side of the primary sensory region, either unilaterally or bilaterally. Endings of secondary afferents branch less and extend for a shorter distance along the

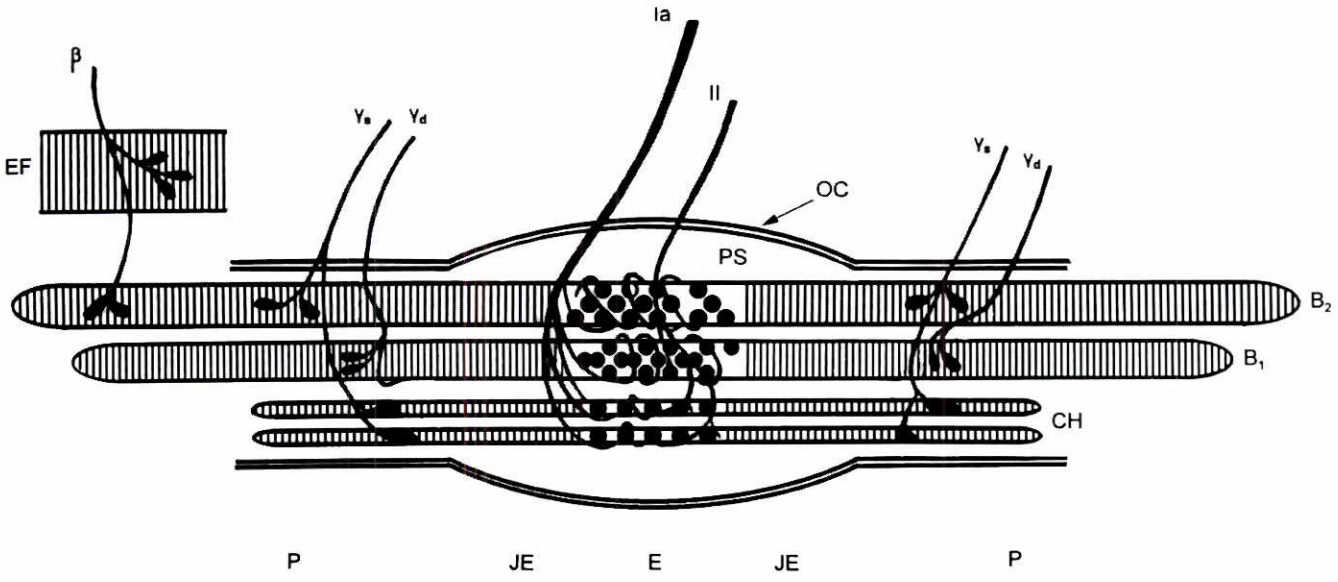

Fig. 1. Diagram showing in longitudinal section the salient features of an adult mammalian muscle spindle. *Nuclear bag$_1$ (B_1), nuclear bag$_2$ (B_2) and nuclear chain (CH) intrafusal fibers are enclosed by a multilayered outer capsule (OC). Intrafusal fibers are innervated by primary or Ia (Ia) and secondary or group II (II) afferents, and by dynamic (d) and static (s) gamma motor or fusimotor neurons. Dynamic and static ß or skeletofusimotor neurons may also be present. These innervate both extrafusal (EF) and intrafusal fibers. For reasons of simplicity, only one generic ß axon is shown. The length of individual intrafusal fibers is divided into an equatorial region (E), and on either side of it, juxtaequatorial (JE) and polar (P) regions. The black dots at the equator of intrafusal fibers are nuclei. At the equatorial region there is an expanded periaxial space (PS) between the outer capsule and the intrafusal fiber bundle.*

length of intrafusal fibers than endings of primary afferents. Most sensory axon contacts occur prior to the formation of the outer spindle capsule (Kucera *et al.*, 1989), and all early afferent contacts are with primary myotubes (Kozeka and Ontell, 1981).

The first sensory terminals are electron dense (Landon, 1972) and already contain vesicles, mitochondria and microfilaments, and they are covered on their external surfaces by basal lamina (Kozeka and Ontell, 1981), but not by Schwann cells, (Landon, 1972). Sensory cross terminals, which are sensory terminals of primary and secondary afferents that supply more than one intrafusal fiber, are common in the young muscle spindle because of the closeness to each other of forming myotubes. In 4-day-old rats nuclear chain fibers may receive all their sensory input via cross terminals. Completion of the adult pattern of sensory innervation is in part accomplished through elimination of cross terminals (Kucera *et al.*, 1988a).

The characteristic appearance of mammalian sensory innervation takes shape gradually. Typical annulospiral endings of primary afferents appear in human muscles in the second half of the fetal period (Mavrinskaya, 1967). In kittens full development of coils of primary endings extends into the first few postnatal weeks, proceeding from an irregular network to evenly spaced spirals (Patak *et al.*, 1992). Similarly, primary afferents in mice do not acquire all of their spirals until PN30 (Osawa *et al.*, 1988) to PN40 (Maeda *et al.*, 1985). Secondary afferents are not recognized in rats until E20, after primary afferents have established contact with intrafusal myotubes (Zelena, 1964; Kucera *et al.*, 1988b). There is some overlap in territory covered by primary and secondary afferents in prenatal rat spindles, as opposed to postnatal receptors, in which they are separated from each other (Kucera *et al.*, 1988a). The initial principal steps in establishing the afferent innervation of the mammalian spindle are illustrated in Figure 2.

Efferent innervation

In rodents, motor terminals are not observed until after the first sensory axons have made contact with intrafusal fibers (Milburn, 1973; Kozeka and Ontell, 1981). In some instances transient beta or skeletofusimotor axons, efferent axons that innervate both extrafusal and intrafusal fibers (Fig. 1), arrive on nuclear bag$_2$ fibers at the same time as the primary afferent (Kucera *et al.*, 1989). As with sensory axons, gamma or fusimotor axons innervate intrafusal fibers in the order in which they are formed, the nuclear bag$_2$ fiber always being the first. From E20 until birth motor terminals in rats are essentially limited to nuclear bag$_2$ fibers (Kucera *et al.*, 1988a), or they may be absent altogether (Landon, 1972). In the neonatal receptor (PN0), approximately four days after the appearance of the primary afferent, efferent innervation extends to nuclear bag$_2$ and to nuclear bag$_1$ fibers. Rat nuclear chain fibers are well supplied with fusimotor axons by about PN4 (Kucera *et al.*, 1988b). In the earliest recognizable fetal cat spindle stages, motor and sensory terminals may have overlapping territories (Milburn, 1984). There is no overlap between sensory and motor endings in rat soleus spindles at any time (Kucera *et al.*, 1988b). Schwann cell extensions cover motor terminals as soon as they are recognized in cat spindles (Milburn, 1984), whereas this does not take place in rats until later (Kucera *et al.*, 1988a), and motor axons in this species are still unmyelinated at PN4 (Kucera *et al.*, 1988b).

Most gamma axons enter the spindle together with sensory fibers at the equator. After penetrating the outer spindle capsule gamma axons separate from afferents to form motor bundles, each

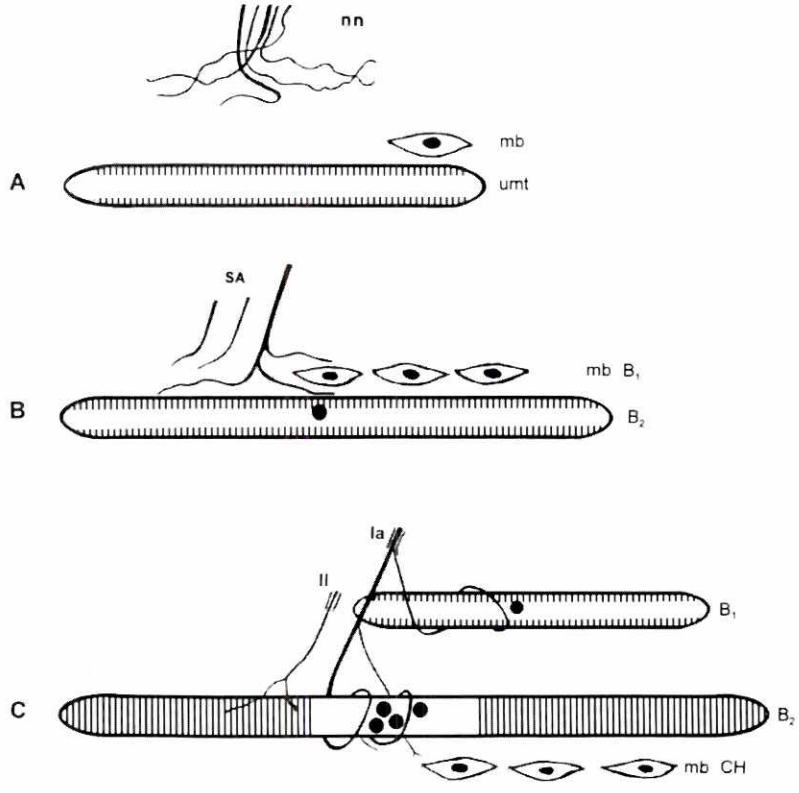

Fig. 2. Schematic representation of major events in the early stages of development of sensory innervation of mammalian muscle spindles. (A) *An unmyelinated network (nn) of thin and thick axons appears and approaches presumptive undifferentiated intrafusal myotubes (umt) and myoblasts (mb).* **(B)** *Several sensory axons (SA) begin to contact myotubes. Myosensory junctions form first between the largest afferent, the presumed primary afferent, and a point on the primary intrafusal myotube that will become the equator of the future nuclear bag*$_2$ *intrafusal fiber. The afferent may also send collaterals to myoblasts. After sensory contact central nuclei (black dot) appear gradually at the equator of the nuclear bag*$_2$ *myotube. Myoblasts align and fuse to form the nuclear bag*$_1$ *intrafusal myotube.* **(C)** *Some sensory axons may retract. Secondary afferents (II) contact myotubes distal to the primary sensory region. Intracapsular portions of axons are still unmyelinated. Endings of primary afferents (Ia) begin to form spirals. The second and third intrafusal myotubes to be innervated by the primary afferent may be the nuclear bag1 and the first nuclear chain, or vice versa, depending on the order in which they are formed.*

bundle containing several axons. Bundles become progressively smaller as they approach the distal pole, due to intermittent termination of axons along the more proximal sections of the polar region. The two poles in a spindle are usually supplied by different motor bundles (Kucera *et al.*, 1988b). At least in rats given gamma axons often project to more than one type of intrafusal fiber to create non-specific innervation. Perinatally nuclear bag fibers become polyneuronally innervated. In the earlier developmental stages nuclear bag$_2$ fibers receive more motor endings than nuclear bag$_1$ fibers, but by PN4 the allocation of endings to the two fiber types is reversed (Kucera *et al.*, 1988a). Chain fibers typically do not acquire more than one motor axon per pole. Because of postnatal longitudinal growth of intrafusal fibers, motor endings are farther removed from the equator in adult spindles than in immature spindles. More motor axons per spindle are found in immature than in adult rat spindles, indicating that retraction of some axons occurred (Kucera and Walro, 1988), possibly due to competition for available myotubes. Degeneration and sprouting of fusimotor and skeletofusimotor axons is seen postnatally in normal cat and rabbit spindles (Barker and Ip, 1966), presumably as a part of a continuous renewal of the motor innervation of spindles.

Genesis of intrafusal fibers and development of myosin heavy chain profiles

It once was thought likely that the final number of intrafusal fibers in rat spindles is obtained through splitting of those myotubes that are present at birth (Marchand and Eldred, 1969). It is now accepted that during normal development all intrafusal fibers form de novo from fusing myoblasts and not by splitting of existing myotubes. Splitting takes place in regenerating intrafusal fiber bundles, and it has been reported to occur in a limited region at the ends of intrafusal fibers during normal development (Cuajunco, 1927), possibly as an adaptation for attachment to connective tissues.

Mature intrafusal fibers are much shorter than extrafusal fibers (Barker, 1974; Kozeka and Ontell, 1981), but in embryonic muscles nuclear bag myotubes may be as long as extrafusal primary myotubes (Kozeka and Ontell, 1981). In perinatal rats the intrafusal myotubes that form nuclear bag$_2$ intrafusal fibers may extend the full length of leg muscles. The later-formed nuclear bag$_1$ and nuclear chain fibers are shorter (Kucera *et al.*, 1988a). Intrafusal myotubes that are formed subsequent to the nuclear bag$_2$ myotube begin to assemble at the nuclear bag$_2$ myotube sensory region (Kozeka and Ontell, 1981), and they then grow distally towards the polar regions (Milburn, 1984; Kucera and Walro, 1990a).

Intrafusal fiber bundle formation starts while intrafusal fiber basal lamina tubes are still incomplete. The nuclear bag$_2$ myotube assembles from myoblasts first. Thereafter, other groups of myoblasts fuse to sequentially form additional intrafusal myotubes. Until recently it had been generally accepted that the remaining fibers arose in the order of nuclear bag$_1$ and nuclear chain. New evidence indicates that in rats, depending on the muscle, the second myotube to be formed may be either a nuclear bag$_1$ or a nuclear chain, suggesting that several components interact to determine intrafusal fiber succession. In such a system an extrinsic factor, perhaps conveyed via the primary afferent neuron, is envisioned to regulate the phenotypic expression of pluropotential myotubes (Kucera and Walro, 1994). Newly forming intrafusal myotubes engage in temporary pseudopodial apposition with existing myotubes, as if to seek structural support during growth

(Landon, 1972; Milburn, 1973, 1984; Kozeka and Ontell, 1981; Kucera *et al.*, 1989). A similar process occurs during extrafusal myogenesis (Duxson *et al.*, 1989). Ultimately, the contiguity of old and new myotubes wanes, and all myotubes form independent fibers with separate basal laminas (Milburn, 1973; Kozeka and Ontell, 1981). Secondary intrafusal myotubes (Milburn 1973, 1984; Kozeka and Ontell, 1981), or all subsequently forming intrafusal myotubes (Milburn, 1984; Kucera *et al.*, 1988a), are apposed to the first-formed nuclear bag_2 fiber. In cat spindles the nuclear bag_1 fiber may be placed aside from the other fibers, and based on this spatial arrangement, the view has been expressed that the nuclear bag_1 fiber may assemble away from the other fibers (Butler, 1979). Near lethal doses of ionizing irradiation given to rat fetuses beginning at the day of birth does not prevent the assembly of the nuclear bag_1 and nuclear chain intrafusal fibers (Bravo-Rey *et al.*, 1969), the two types of fiber which appear perinatally and during the first postnatal week, respectively.

From observations that the first-formed nuclear bag_2 myotubes and large axons appear concurrently evolved the concept that differentiation of the intrafusal fiber bundle is induced by sensory axons (Tello, 1922; Zelena, 1957, 1964; Milburn, 1984; Kucera *et al.*, 1988a, 1989), perhaps via a trophic factor. Somata of sensory axons that project to muscle spindles are immunoreactive for neurotrophin-3 (Zhou and Rush, 1995), a growth factor. Neurotrophin-3 mRNA is expressed in normally developing and adult rat nuclear bag intrafusal fibers (Copray and Brouwer, 1994). In homozygous mutant mice that lack neurotrophin-3 the number of sensory (Ernfors *et al.*, 1994) and fusimotor neurons (Kucera *et al.*, 1995a,b) is severely reduced, or they are altogether absent. No spindles or Golgi tendon organs develop and affected animals die shortly after birth (Ernfors *et al.*, 1994). In heterozygotes the number of fusimotor neurons (Kucera *et al.*, 1995b) and of muscle spindles (Ernfors *et al.*, 1994; Kucera *et al.*, 1995b) are reduced to about 50 % of normal. The population of beta axons is only slightly reduced (Kucera *et al.*, 1995a). As neurotrophin-3 is not found in extrafusal fibers, but is present in nuclear bag intrafusal fibers (Copray and Brouwer, 1994), in somata of large sensory neurons which quite likely are spindle afferents (Zhou and Rush, 1995), and in fusimotor neurons, it has been proposed that neurotrophin-3 is instrumental in the development and maintenance of muscle spindles (Kucera *et al.*, 1995a,b; Zhou and Rush, 1995). Most large muscle afferents express tyrosine protein kinase receptor encoded by the *trkC gene*. The kinase is the preferred receptor for neurotrophin-3. Most cutaneous afferents lack the receptor. It is likely that during the period of normal cell death the interaction of neurotrophin-3 with its receptor sustains spindle afferents (Klein *et al.*, 1994; Oakley *et al.*, 1995). Collectively, these results illustrate the effectiveness of molecular biology as a tool in studying muscle spindle genesis.

Some studies have been undertaken to explore how sensory-motor connections in the spinal cord are determined. Results presented by Wenner and Frank (1995) provide evidence that during development sensory feedback from muscles specifies that spindle afferents connect with alpha motor neurons that project to muscles in which the feedback signal originated. Developing spinal cord secretes a factor, probably the axon guidance molecule semaphorin III, that restricts the synapsing of non-proprioceptive afferents to the dorsal horn of the spinal cord, but permits neurotrophin-responsive cells, such as spindle afferents, to make contact with alpha motor neurons in the ventral horn of the spinal cord (Messersmith *et al.*, 1995). Although much additional work is required in this area, it can be already said that neurotrophic molecules are necessary for establishing peripheral and central connections of muscle afferents and efferents.

Despite its potential for obtaining useful data, the study of spindle development by *in vitro* methods has been rarely attempted. In cultures containing pieces of human intrafusal fibers from biopsied muscles, multinucleated cells reminiscent of developing intrafusal fibers are apparent from 20 to 70 days after placing explants into culture (Elliott and Harriman, 1974), demonstrating that intrafusal fibers can regenerate in isolated systems. No coculturing of neuronal elements was mentioned in this study. If no afferents or extracts from afferents were present, then local environment should play a role in spindle induction. The relationship between appropriateness of targets and the behavior of sensory growth cones has been thoroughly examined by Woodbury and Scott (1995). They were able to grow in culture separate populations of cutaneous and muscle afferents. This was possible because of the discontinuous location in the trigeminal system of cutaneous afferents (trigeminal ganglion) and muscle afferents (mesencephalic nucleus). Cutaneous neurites grew into epidermal explants, but then retracted; muscle afferents, on the other hand, stopped growing when encountering epidermal explants, but did not retract. When exposed to myotubal cultures, on average muscle afferent growth cones remained in contact with myotubes longer than did cutaneous afferent growth cones. These contrasting behaviors illustrate that different groups of sensory neurites can respond differently to specific environmental conditions, that is, they can recognize foreign as well as suitable substrates. It then appears that environmental cues are one factor in guiding innervating axons along specific pathways to their proper targets, as for example, primary afferents to presumptive muscle spindles. One word of caution is in order. These results have been obtained in regenerating tissue, and it has yet to be proven that similar processes occur in native tissues.

Early effects of contact by an afferent axon with the undifferentiated nuclear bag_2 myotube causes it to enlarge slightly and to enclose within its basal lamina tube sensory terminals (Milburn, 1984). Later there is an increase in central intrafusal nuclei and a loss of cross striations at the sensory region (Cuajunco, 1927, 1940; Zelena, 1957, 1964; Milburn, 1984). For some specialized muscles spindle induction by afferents may not hold, as in rat tail muscles, in which intrafusal fibers can be recognized prior to the arrival of sensory axons (Ovalle, 1976).

Data on MHC composition of intrafusal myotubes, and later myofibers, are available from the time of their first recognition (Pedrosa-Domellöf, 1991; Kucera *et al.*, 1992). The nuclear bag fiber-specific slow-tonic MHC has been identified in heterogeneous populations of primary myotubes of humans and rats. One interpretation of the early presence of this isoform has been that it is an intrinsic property of a specific cell lineage (Thornell *et al.*, 1989). Yet, slow-tonic MHC is not fully expressed in nuclear bag fibers until myosensory junctions mature, and hence, its expression may be governed by sensory axons (Kucera *et al.*, 1989; Kucera and Walro, 1990a,b). One suggested mechanism by which sensory innervation can regulate MHC phenotype is through control of genes in intrafusal nuclei at the equator (Kucera and Walro, 1989a). The inductive capacity of the primary sensory axon

in this perceived system is strongest at the equator with its accumulation of nuclei, and it declines as nuclear density decreases towards the juxtaequator and the pole. A decreasing sensory regulatory influence on myonuclei could explain the regional variation in MHC expression seen for some isoforms in specific intrafusal fiber types when going from the equator to the pole (Kucera and Walro, 1988, 1989a,b; Maier *et al.*, 1988; Pedrosa and Thornell, 1990; Pedrosa *et al.*, 1990).

It has been proposed that in rats both extrafusal and intrafusal fibers arise from a common bipotential precursor, because MHC profiles of intrafusal myotubes in the early spindle resemble to some degree those of extrafusal myotubes (Kucera and Walro, 1990a). This is most apparent when comparing precursors of nuclear bag_2 intrafusal fibers with type I extrafusal fibers (Kucera and Walro, 1995; Walro and Wang, 1995). Subsequent differences in MHC profile between extrafusal and intrafusal fibers, and among intrafusal fibers, are thought to arise through a combination of inherent properties of fibers and the actions of afferents. Others (Pedrosa and Thornell, 1990; Soukup *et al.*, 1995) have suggested that immunostaining for MHC between extrafusal and intrafusal myotubes is too diverse to support the notion of a common precursor. Further support for separate pools comes from the finding that supernumerary nuclear bag fibers and nuclear chain fibers in neonatally deefferented rat spindles derive from at least two different types of intrafusal satellite cells (Soukup *et al.*, 1993). One problem with the sensory axon induction model is that no slow-tonic MHC is observed in nuclear chain fibers. In its defence it can be said that afferent inductive capacities may decline over time, and then are insufficient to induce the slow-tonic isoform in the late-appearing nuclear chain fibers. Declining inductive powers could also explain why the intensity of immunostaining at the equator is not the same for all members of the slow MHC family (Kucera and Walro, 1989a; Pedrosa *et al.*, 1990).

TABLE 1

LANDMARKS IN MAMMALIAN OUTER CAPSULE DEVELOPMENT

First appearance to birth

- Delicate sheath, perhaps of connective tissue origin[1]
- Ring of fibroblasts in region of future outer capsule[2]
- Capsule is an incomplete cover that is continuous with the layer that covers the spindle nerve; there is no basal lamina[3]
- Thin sheath of fibroblasts and Schwann cells surrounds the primary intrafusal myotube[4]
- Primitive capsule from extensions of perineural cells[5]
- Early bilamellate capsule in sensory region from perineural epithelium and fibroblasts[6]
- Accumulation of extracellular connective tissue[2]
- Capsule consists of several layers of flattened cells[4]
- Multilayered capsule[7]
- Capsule grows from equator towards the pole; perineural nature of capsule becomes more apparent[3]

Early postnatal period

- There are several layers of perineural cells[8]
- Collagen and elastin fibrils form networks in outer capsule[4]
- There are now basal laminas associated with outer capsule cells[9]
- Previously small or absent periaxial space becomes prominent at sensory region[10]

[1]Cuajunco, 1927; [2]Swatland, 1974; [3]Landon, 1972; [4]Milburn, 1984; [5]Kozeka and Ontell, 1981; [6]Milburn, 1973; [7]Frequent mentioning; nature of layers not always specified; [8]Kucera *et al.*, 1988b; [9]Dow *et al.*, 1980; [10]common observation.

As has been described for extrafusal fibers (Whalen *et al.*, 1981), there is in developing intrafusal fibers a succession of MHC isoforms. The best known sequence is the complex pattern observed in rat spindles. In nuclear bag_2 myotubes slow-tonic MHC appears prenatally shortly after afferent contact, and it remains expressed in the mature fibers. Fast-twitch MHC is also present in nuclear bag_2 myotubes prenatally and fibers postnatally. This is in contrast to nuclear bag_1 myotubes in which fast-twitch MHC is expressed prenatally, but not postnatally. Strong immunostaining for slow-tonic MHC appears in nuclear bag_1 fibers at about birth, and remains a permanent feature of this fiber type. Nuclear chain fibers express fast-twitch myosin as soon as they form, while slow-tonic MHC is never expressed. Slow-twitch MHC is recognized in all three fiber types during development, but it as absent from all in the adult (Kucera and Walro, 1990b). An anti-pectoral myosin occurs transiently in neonatal rat nuclear bag_1 and nuclear bag_2 fibers, while an unspecified heart myosin is not expressed in nuclear bag_1 fibers until after birth (teKronnie *et al.*, 1982). In the same species, α-cardiac myosin is present in nuclear bag_2 fibers at E21, and in nuclear bag_1 fibers at PN3, and it remains expressed in both fiber types into adulthood (Pedrosa *et al.*, 1990).

Histochemical changes are observed along with evolving MHC profiles. Intrafusal fiber types as recognized with histochemical reactions for phosphorylase and glycogen appear in mice trunk muscles already prenatally (Wirsén and Larsson, 1964). Fiber type-specific myosin ATPAse profiles are already recognized in nuclear bag and nuclear chain fibers of neonatal kittens (Maier and Eldred, 1974), but in rat soleus mature myosin ATPase profiles are not apparent until PN6 (Kucera and Walro, 1989b), and in rat tail muscles not until PN9 (Ovalle, 1976).

Inhibition of contractile activity has been employed as a tool to study genesis of intrafusal fibers. Administration via the amniotic cavity of the action potential blocker tetrodotoxin to fetal rats at E16, before the spindle anlage forms, does not prevent the initial development of the muscle spindle, such as the appearance of the nuclear bag_2 fiber and a thin spindle capsule, both characteristic features of the receptor at E18. When examined at E21, however, most of the spindles still contain only one intrafusal fiber, a nuclear bag_2. There is also a deficient sensory apparatus and no sign of motor innervation. By then during normal development formation of the nuclear bag_1 fiber would have begun. Despite this deficiency in the number of intrafusal fibers, slow-tonic MHC is expressed. Based on these observations it has been concluded that the inhibition of action potential and subsequent muscular activity due to the presence of tetrodotoxin does not abolish spindle formation, but affects its subsequent differentiation and maturation (Kucera and Walro, 1991a). One other example of how modulation of muscular activity affects receptor development has been demonstrated in mastication. Chewing fine-grained food with less muscular force than normal will lead in jaw closing muscles of young mice to small and incomplete primary afferent endings (Maeda *et al.*, 1988a,b).

Outer and inner capsules

The outer spindle capsule is a fusiform-shaped sleeve that surrounds the intrafusal fibers. It is generally agreed that the

TABLE 2

SOME PHYSIOLOGICAL PARAMETERS OF YOUNG KITTEN SPINDLES

Static and dynamic fusimotor effects on spindle afferent discharge apparent by PN5[1]
Lower afferent firing rates relative to adult animals[1,4]
Small dynamic afferent responses after infusion of succinylcholine[2]
Rapidly adapting static response[3,4]
Maintained static response if combined with fusimotor stimulation[1]
Dynamic response matures slower than static response[5]
No strict correlation between morphology of sensory endings and physiological response[6]

Properties of afferent signals have been related to structural immaturity of, and to structural changes occurring in, kitten spindles. [1]Gregory and Proske, 1986; [2]Gregory and Proske, 1987; [3]Skoglund, 1960; [4]Gregory and Proske, 1985; [5]Jami *et al.*, 1989; [6]common observation.

capsule starts as a single layer at the sensory region after afferent contact, and then grows distally towards both poles. In E19 mice it is only about 1/5 the length of the intrafusal fiber bundle (Kozeka and Ontell, 1981). Cuajunco (1927) thought that the delicate membrane-like early outer capsule was formed from inner perimysium. Current data indicate that in adult receptors it is composed of perineural epithelium, or perineural epithelium-like cells, and of cellular and acellular components of connective tissue. The perineural epithelium usually does not enclose the more distal portions of intrafusal fibers, but endomysial and perimysial components continue on to the ends of fibers. In rats, capsular growth is initiated by processes of cells that arrive with, and provide covers for, the nerves that innervate spindles (Landon, 1972). In mouse receptors the first layer of perineural epithelial cells is recognized at E17 (Kozeka and Ontell, 1981), and in mice (Kozeka and Ontell, 1981) and in rats (Kucera and Walro, 1990b) multiple epithelial layers appear by E19. With time the capsule cells flatten and they acquire a prominent network of endoplasmic reticulum. Capsular collagen and elastin fibrils are not seen until 2-3 weeks postnatally in rats (Landon, 1972), but collagen is already associated with the capsule in the late fetal period in cats (Milburn, 1984). Outer capsules in cat peroneus longus muscles begin as a single sheath of Schwann cells and associated fibroblasts (Milburn, 1984). Thus, in cat fetuses the early capsule is already a collection of connective and other tissues. By the time all intrafusal fibers have been assembled, the capsule has several layers of flattened cells of the same kind as those that ensheath the spindle nerve (Milburn, 1984), and which attach to each other by junctional complexes (Landon, 1972). These connections are a likely first step in establishing a spindle-extracellular space barrier. There are no basal laminas in the earliest outer capsule (Landon, 1972), but in adults each layer of flat outer capsule cells is associated with a basal lamina (see Barker, 1974). Major events in outer capsule development are summarized in Table 1.

Inner capsules are probably inward extensions of outer capsule cells. They grow towards the center of the receptor and curl around individual intrafusal fibers, in effect separating them from one another. Nascent inner capsules are seen during the first postnatal week in mice (Kozeka and Ontell, 1981) and in rats (Milburn, 1973).

The outer surface of the growing outer capsule is continuous with the endomysium of the extrafusal space. The inner surface of the outer capsule is in direct contact with the intrafusal fiber bundle, and thus, at first there is no periaxial space. To date no definite experimental evidence is available on which forces cause the outer capsule to bulge at the sensory region to create the periaxial space. As a rule, the space becomes noticeable after all intrafusal fibers have been formed. This takes place in cat peroneal (Milburn, 1984) and gastrocnemius (Maier and Eldred, 1974) muscles during the first to second postnatal weeks. In rat soleus, in which the full complement of intrafusal fibers is not reached until PN4, there is a well-formed capsule at this age, but still no distinct periaxial space (Kucera *et al.*, 1988b).

Physiological development

A number of studies have monitored the maturation of the afferent spindle discharge in kittens and discussed how it compares to structural correlates (Table 2). Overall, the precise connection between physiological responses and morphological substrates is not easily made (e.g. Vejsada *et al.*, 1988). Stretch receptor-like signals in response to vibration stimuli can be recorded from spindles as early as two days after birth (Gregory and Proske, 1988) when branching patterns of the primary afferent are still immature. Thus, complete arborization is not a prerequisite for afferent signals to issue from developing receptors. The major difference between the afferent signal of very young and older kittens in response to ramp-and-hold stretches is one of amplitude of the sensory signal, increasing progressively with increasing age (Patak *et al.*, 1992). Conduction velocities for both fusimotor and skeletofusimotor axons increase continuously in 1-23 day-old kittens, but much more rapidly for skeletofusimotor axons than for fusimotor axons. Increases in conduction velocity have been attributed to gradual myelinization of axons (Gregory and Proske, 1986). Effects of fusimotor and skeletofusimotor inputs on spindle discharge can be already detected during the first three postnatal weeks in kittens (Gregory and Proske, 1985). Moreover, separate static and dynamic fusimotor actions on afferent discharges can be recorded as early as PN5 in most kitten spindles, even though firing rates are low (Gregory and Proske, 1986). It has been suggested that the lack of response of the primary afferent to fusimotor stimulation in neonatal kittens may as much be due to functional immaturity of afferents than of efferents (Gladden and Milburn, 1988). The dominant response to muscle stretch in very young kittens is dynamic; static discharges are not maintained until the second postnatal week (Skoglund, 1960; Jami *et al.*, 1989). Despite its prevalence in the early postnatal period, the dynamic component of the afferent response matures slower than the static component (Jami *et al.*, 1989), and the sustained tonic effect after the administration of succinylcholine is lacking (Skoglund, 1960; Gregory and Proske, 1987). Perinatally only nuclear bag_2 fibers may contribute to the dynamic peak of the afferent signal, because of structural immaturity of the later arriving nuclear bag_1 fibers and their neural connections, the units most likely responsible for producing the bulk of the dynamic component of the afferent signal in adults (see Boyd, 1976). Kitten spindles are more length-sensitive under dynamic conditions than adult receptors (Gregory and Proske, 1988), perhaps due to an incomplete lattice of elastic fibers at the equator; however, kitten spindles have a greater threshold to vibration than adult receptors, which does not significantly change even in the presence of succinylcholine (Gregory and Proske, 1987).

Extrafusal and intrafusal fibers are of approximately the same size in newborn kittens (Maier and Eldred, 1974), but when stimulating single motor axons, only extrafusal fibers develop measurable tension (Gregory and Proske, 1991). Reasons for this difference probably are: 1) that single alpha motor axons innervate more extrafusal fibers than single gamma motor axons innervate intrafusal fibers; 2) an immature intrafusal neuromuscular junction; and 3) the failure of most myofibrils to extend uninterrupted from one pole across the equator to the other pole.

Regeneration

Degeneration-regeneration has been used as a model for studying spindle development. The two principal methods employed are nerve crush and nerve sectioning. In nerve crush the basal lamina is preserved, and regeneration is point-to-point. Bisecting the nerve leads to severe deficits, random reinnervation, and a longer regeneration period.

Nerve crush in rat leg muscles induces deficiencies in the number of spindles and in the number of intrafusal fibers if it is performed between PN0 and PN6 (Werner, 1973a), or produces only occasional atypical spindles (Hnik and Zelena, 1961). Prenatal (Zelena, 1957), neonatal or postnatal (Zelena and Hnik, 1960; Schiaffino and Pierobon-Bormioli, 1976; Walro *et al.*, 1991) nerve section with or without devascularization causes rat intrafusal fibers to degenerate or show atypical features. In adult rat lumbricals motor and sensory endings disappear three to five days after nerve section, respectively (Yamamura and Schober, 1982). Microscopic changes occurring as a result of denervation include decreases in the sizes of intrafusal fibers and their mitochondria (Schröder, 1974a; Schröder *et al.*, 1979), and supernumerary (Schröder, 1974a; Schober and Yamamura, 1983) and irregularly arranged (Schröder, 1974b) basal laminas.

Cat soleus muscles regenerating after nerve section contain about two-thirds of the usual number of spindles. Of these, 50-60% become reinnervated. Most reinnervated spindles show abnormal sensory endings. Some of the reinnervating sensory axons form fine networks, not unlike those that mark the beginning of spindle development; however, for the most part, configurations observed during regeneration are not identical to those that occur during normal development (Ip *et al.*, 1988; Ip and Vrbova, 1973). In rat muscles in which the sciatic nerve is crushed, abnormal regenerated sensory and motor endings are still frequently encountered 3 months after the lesion. If the nerve is sectioned instead of crushed, there are more anomalous sensory configurations (Dieler and Schröder 1990a) and regeneration takes longer (Dieler *et al.*, 1992), indicating that intact endoneural sheaths and basal laminas facilitate the reestablishment of neuromuscular contacts.

Nerve crush (Schröder, 1974a) and deefferentation (Kucera and Walro, 1988; Soukup *et al.*, 1993; Zelena and Soukup, 1993; Novotova and Soukup, 1995) lead to an increase in the number of intrafusal fibers per spindle. This gain is caused by a combination of splitting of existing fibers and the formation of new fibers from precursor cells (Schröder, 1974a; Soukup *et al.*, 1993; Zelena and Soukup, 1993) or primarily by neomyogenesis (Novotova and Soukup, 1995). Neonatal deefferentation of rat leg muscles has been shown to lead to a mild atrophy of intrafusal fibers (Zelena and Soukup, 1973). Adult guinea pig nuclear chain fibers are more atrophied than nuclear bag fibers four weeks after nerve section, demonstrating specific responses between fiber types (Maier *et al.*, 1974). Differential responses are also noted in ischemic rat spindles where nuclear bag fibers degenerate and regenerate quicker than nuclear chain fibers (Diwan and Milburn, 1986).

Intact innervation is a requirement for complete spindle morphogenesis. Rats which receive intraperitoneal injections of the neurotoxin ß-bungarotoxin at E16 or E 17 display no intramuscular nerve branches or spindles in E21 muscles (Kucera and Walro, 1990c). Yet, in reinnervated and non-reinnervated grafts (Rogers and Carlson, 1981; Walro *et al.*, 1991) the initial stages of regeneration occur even in the absence of sensory endings. Successful reinnervation may hinge in part on the outer capsule remaining intact (Rogers and Carlson, 1981). Rat medial gastrocnemius muscles allowed to regenerate after neonatal nerve crush contain hybrid fibers whose morphology is typically extrafusal on one pole and intrafusal on the other. This arrangement can be thought of as evidence that early myotubes have the potential to differentiate into extrafusal or intrafusal fibers (Werner, 1973b), and that they derive from a bipotential common stem cell.

In nerve-intact rat extensor digitorum longus muscles which are regenerating after interruption of their vascular supply, muscle spindles with and without sensory innervation are found. In these muscles non-reinnervated intrafusal fibers contain some fast-twitch MHC, but slow-tonic MHC is only observed in intrafusal fibers with sensory innervation (Cui and Walro, 1989). Intrafusal fibers regenerating in denervated grafts express neonatal and fast-twitch, but not slow-twitch and slow-tonic MHC (Walro *et al.*, 1991). On the other hand, slow myosins are expressed in regenerating nerve intact grafts. Hence, innervation is necessary for the complete intrafusal fiber-specific MHC profile to be expressed. Rat muscles regenerating for 30 days after neonatal nerve crush, and which become reinnervated by afferents only, will contain some intrafusal fibers that express slow-tonic MHC. The presence of this isoform in this preparation has been taken as evidence that it is the afferent component which regulates MHC expression, and that afferents maintain inductive powers beyond the normal developmental period (Kucera and Walro, 1992a).

The importance of the primary afferent to normal spindle development has been repeatedly demonstrated by selectively eliminating the sensory or motor supply. Deafferentation in rats during the first postnatal week will cause the developing receptor to degenerate, become arrested in its development, or show many anomalous features. Nearly twelve months later there is no periaxial space and only a thin outer capsule (Kucera and Walro, 1987). Distribution of myofibrils and myosin ATPase staining in deafferented adult rat intrafusal fibers after grafting is more characteristic of extrafusal fibers than of intrafusal fibers (Walro *et al.*, 1989). *De novo* supernumerary spindles form in rat muscles after neonatal nerve crush if exogenous nerve growth factor is administered concurrently (Sekiya *et al.*, 1986). The same result is obtained when regenerating muscles that receive exogenous nerve growth factor are permitted to become reinnervated by afferents only (Kucera and Walro, 1992a; Kucera *et al.*, 1993a). It is thought that the additional spindles are induced through contact of undifferentiated myotubes with new afferent collaterals that sprouted in response to increased levels of nerve growth factor (Sekiya *et al.*, 1986). Even though boosted by the additional nerve growth factor, the results illustrate the inductive potency of afferents. Receptors in regenerating muscles that receive afferents but no efferents may be deficient in intrafusal fiber content (Kucera and Walro, 1992a;

Kucera *et al.*, 1993a) and often lack nuclear bag_2 fibers (Kucera *et al.*, 1993b). Rather than to a decline in afferent potency, the nuclear bag_2 deficit has been attributed to a lack of myoblasts and myotubes available for afferent induction. The frequent absence in denervated muscles of nuclear bag_2 and sometimes also of nuclear bag_1 fibers, coupled with the presence of nuclear chain fibers (Kucera *et al.*, 1993a), indicates that neither one of the nuclear bag fiber types is a required template for the assembly of nuclear chain fibers (see Milburn, 1973 and Fig. 1 therein).

Results of experiments on deafferentation and deefferentation have been interpreted (Kucera and Walro (1988, 1992b) to mean that by and large motor innervation plays no significant role in establishing the MHC composition of intrafusal fibers; however, there is some evidence that afferent induction cannot account for all variations in immunostaining along the length of intrafusal fibers, and that full MHC differentiation requires the presence of both sensory and motor innervation (teKronnie *et al.*, 1982; Walro *et al.*, 1991). Based on regional staining in the striated portions of intrafusal fibers after neonatal deefferentation of rodent muscles, similar but lesser regulatory properties than those assigned to afferents have been proposed for motor axons (teKronnie *et al.*, 1982; Soukup *et al.*, 1990; Pedrosa-Domellöf *et al.* 1991). Rat nuclear bag_2 fibers retain a heart myosin isoform after neonatal deefferentation which normally is downregulated by PN21 (te Kronnie *et al.*, 1982). In neonatally deefferented rat spindles MHC profiles will not fully differentiate (Soukup and Zelena, 1985). The postnatal fading of slow-tonic MHC along the encapsulated polar region of nuclear bag_2 fibers fails to take place, and slow-twitch MHC is expressed in the B region on either side of the equator in nuclear bag_1 fibers, from which it is normally absent (Soukup *et al.*, 1990). This redistribution causes an approximation in MHC profile of the two types of nuclear bag fiber. Myosin heavy chain content of the nuclear chain fibers are not affected by deefferentation (teKronnie *et al.*, 1982; Kucera and Walro, 1988; Soukup *et al.*, 1990; Pedrosa-Domellöf, 1991). Despite the changes caused by deefferentation, in most instances it does not prevent classification of fibers into nuclear bag_1 and nuclear bag_2 types (Zelena and Soukup, 1974; teKronnie *et al.*, 1982; Kucera and Walro, 1988).

Intact innervation is also a requirement for the maintenance of normal histochemical profiles. Four weeks after nerve section succinate dehydrogenase activity is greatly reduced in adult guinea pig nuclear chain fibers. Under the same conditions, alkaline-stable myosin ATPase activity increases in nuclear bag_1 fibers and decreases in nuclear bag_2 fibers (Maier *et al.*, 1974) to cause an approximation of fiber type profiles. Neonatal deefferentation of rat leg spindles lowers intrafusal myosin ATPase in general (Zelena and Soukup, 1974). In rat soleus muscle spindles deafferented for 12 months, the encapsulated portions of nuclear bag fibers reverse their myosin ATPase activity and assume the characteristic profile of their extracapsular portions (Kucera, 1980). Myosin ATPase staining of intrafusal fibers in reinnervated rat medial gastrocnemius after nerve crush and periodic administration of nerve growth factor during recovery is similar to that of normal intrafusal fibers (Kucera and Walro, 1989c).

Despite grossly abnormal morphology, most spindles in regenerated muscles respond with a discharge to ramp-and-hold stretch (Quick and Rogers, 1983; Barker *et al.*, 1986; Palecek *et al.*, 1989), even though maximum firing rates are generally lower than normal (Barker, *et al.*, 1986; Palecek *et al.*, 1989), and there may be no or an abortive discharge during the held phase of the stretch (Hyde and Scott, 1983; Quick and Rogers, 1983). In cat peroneus brevis maximum firing rates of primary afferents do not fully recover, even four months after nerve crush (Barker *et al.*, 1986), while the basic morphological features of sensory endings become reestablished sooner (Barker *et al.*, 1985). Cat spindles regenerating after devascularization display faulty sensory endings, but they will respond nearly normal to a 2 mm ramp-and-hold stretch (Barker and Scott, 1990). From these data it may be concluded that there is no strict relationship between appearance and function of sensory endings. Nevertheless, when comparing the extremes of poorly (1 intrafusal fiber/spindle) versus well restored (4 intrafusal fibers/spindle) rat spindles, there is a good correlation between afferent structure and function after nerve crush, that is, the closer to normal the structure, the closer to normal the pattern of afferent discharge (Palecek *et al.*, 1989). An increase in elastic fibrils around intrafusal fibers may in otherwise anomalous receptors sustain pliability of the sensory region, and hence, aid in the generation of fairly typical signals (Dieler and Schröder, 1990b).

Reinnervation of polar regions and its effect on afferent discharge has been also examined. After neonatal nerve crush motor endings become reestablished, but there are much fewer endings than in normal spindles, and those that are present are more like endings of alpha axons than of gamma axons. This paucity of motor endings has been related to a greater susceptibility of immature efferents to axotomy compared to afferents, and perhaps to difficulties in advancing along a damaged afferent trajectory to the spindle (Kucera *et al.*, 1993a). No large difference is seen in the percentage of beta motor axons reinnervating cat leg muscle spindles after 6-7 weeks of nerve crush or section; however, there are more static beta axons in reinnervated receptors than in normal spindles (Scott, 1987). Six months after nerve section many more beta motor axons innervate cat nuclear bag_2 fibers than normally (Barker *et al.*, 1995), perhaps because of an accompanying decrease in Ia connections (Banks and Barker, 1989) and a diminished afferent control over spindle development. Elimination of motor input from spindles through neonatal deefferentation has no significant effect on passive afferent discharge (Hnik *et al.*, 1977). Even several months after deefferentation afferents still produce a slowly adapting signal in response to maintained stretch.

Repeated injections of lidocaine into masseter muscles of young mice result in smaller diameter primary sensory endings (Maeda *et al.*, 1989). Necrosis is seen at the equatorial regions of rat nuclear bag fibers after intramuscular injection of bupivacaine (Milburn, 1976), leading to the destruction of equatorial nuclei. Most sensory fibers also undergo necrosis, while fusimotor fibers are less affected. Nuclear bags and nuclear chains are absent, as are afferent spirals. In regenerating spindles of this model the primary afferent may have lost the capacity to induce equatorial nucleation, which in turn may have prevented maturation of sensory endings and terminals (Milburn, 1976). Loss of equatorial nuclei in sensory denervation has been also reported by Swash and Fox (1974) for human spindles. Thus, most likely, sensory innervation is required for the production and maintenance of equatorial nuclear bags and chains.

Tenotomy has been employed to test for the importance of maintained tension on the development of muscle spindles. Prenatal or neonatal tenotomy of rat gastrocnemius and plantaris muscles has no effect on receptor number and, except for a significant

reduction in length (Zelena, 1963), none on structure. Cutting the soleus tendon in adult rats leads to some degenerative changes in intrafusal fibers (Matsumoto and Baker, 1987). Histochemical profiles of intrafusal fibers are minimally affected (Jozsa *et al.*, 1988). Primary afferents of spindles in tenotomized adult cat medial gastrocnemius respond to muscle stretch with a decreased length sensitivity (Yellin and Eldred, 1970). Intrafusal fibers in these muscles are contorted and shortened, which most likely is the cause of the decreased sensitivity of the receptors. As prenatal and neonatal tenotomy also results in shortened receptors (Zelena, 1963), it may be assumed that their firing rates are also reduced.

Birds

Normal development

General morphology

The structure of avian spindles is less well known than that of mammalian spindles. Like their mammalian counterparts, avian receptors consist of an intrafusal fiber bundle that is protected by an outer capsule and receives sensory and motor innervation. One difference between the two vertebrate classes is that at the equator bird intrafusal fibers are surrounded by an elaborate connective tissue matrix, the most conspicuous component being the collagenous perifibril sheath. It overlies the sensory terminals, and when viewed in cross section, is shaped like a crescent (James and Meek, 1973; Hikida, 1985; Maier, 1992a) (Fig. 3). The size of the axial bundle is much more variable in birds than in mammals, containing from as few as 1 to as many as 16 intrafusal fibers (Maier, 1983). In postnatal muscles three fiber types are recognized at polar regions after incubation with monoclonal antibodies against slow MHC (Maier and Zak, 1990). Fibers in this preparation react either strongly or moderately, or they are unreactive. Hence, using the respective first letters indicating their reactivity for slow MHC, they are designated as S, M or U fibers. Fibers of the U category react with a monoclonal antibody against neonatal/fast MHC and, thus, are considered to be faster contracting than S and M fibers (Maier, 1992b).

Virtually all information on avian spindle development has been collected from chicken muscles. The extensive work of Tello (1922) provides the earliest reliable account. Besides specific data on chicken muscle spindle development, his monograph also contains much basic information on receptor genesis in general, and was influential in shaping the concept that sensory axons induce spindle formation.

Afferent innervation

Chickens hatch after 21 days of incubation, and according to Tello (1922) undifferentiated myotubes in chicken leg muscles may be contacted by presumptive sensory axons as early as E11; however, in subsequent examinations (Rebollo and DeAnda, 1967; Toutant *et al.*, 1981; Maier, 1993a) spindle anlagen could not be pinpointed earlier than E13, and even then their identification was tenuous. Sensory ramifications at E11-13 have been described (Tello, 1922), but probably are not common in this early period. Instead, more often an undivided, unmyelinated parent sensory axon runs along the future equatorial region or curves partially around the primary intrafusal myotube (Maier, 1993a). First order branches of the sensory axon may approach myotubes perpendicularly and clasp them in a pincer-like fashion. By E14

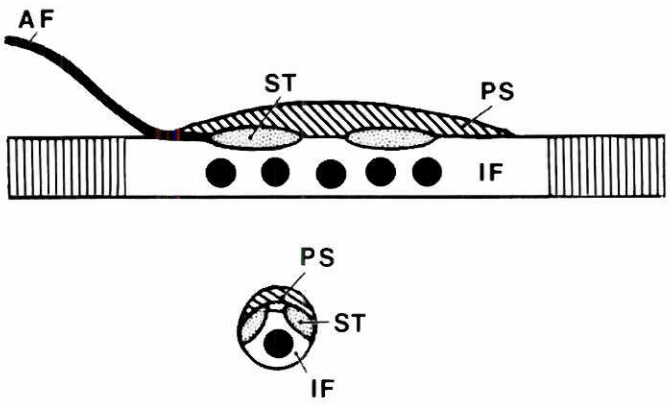

Fig. 3. Diagrammatic representation in longitudinal (top) and cross (bottom) section of the major features at the equator of one adult chicken intrafusal fiber. *Unlike mammals, there are no differences in general equatorial morphology among bird intrafusal fibers (IF). Striations (vertical lines) become indistinct at the equator. Sensory terminals (ST) that arise from endings of large-size afferents (AF) are covered by the collagenous perifibril sheath (PS). In cross section the sheath appears as a crescent. Equatorial intrafusal nuclei (black dots) are largely placed in single file. Nuclear accumulations typical of mammalian nuclear bag fibers are not observed.*

there may be abundant arborization (Tello, 1922), and by E17 much of the basic postnatal branching pattern has become established (Maier, 1993a). Sensory axons in E15 spindle nerves are still mostly unmyelinated, even though Schwann cells are present (Toutant *et al.*, 1981). Histochemically assessed strong acetylcholinesterase activity is found at the sensory region of the equator at E15-16, but it declines before hatching (E21) and gradually disappears during the early postnatal period (Mumenthaler and Engel, 1961). Immunohistochemically, acetylcholinesterase activity is still detected in 8-week-old chickens at the equator, but opposite the myosensory junction (Maier, 1995a). Contrary to mammals, no nuclear bags form at the equator of intrafusal fibers after afferent contact. Instead, and regardless of MHC-based types, large spherical nuclei that are placed in single file are visible in each fiber (Fig. 3). As the myosensory junction matures, a specialized synaptic basal lamina develops that immunostains strongly for the laminin β_2 chain and chondroitin sulfate proteoglycan. At E17 the perifibril sheath is first observed as it begins to cap the sensory basal lamina and the underlying sensory terminals (Maier and Mayne, 1995).

Efferent innervation

In E15 chicken posterior latissimus dorsi muscles motor endings with cytoplasm darker than that of sensory endings are observed at the juxtaequator (Toutant *et al.*, 1981). In postnatal spindles that have been reacted with α-bungarotoxin or an antibody against acetylcholinesterase, motor endings are seen to reach the equatorial-juxtaequatorial junction (Maier, 1989). Motor endplates are first identified in chicken gastrocnemius at E14 and become distinct at E16 (Rebollo and DeAnda, 1967). In developing tibialis anterior and extensor digitorum longus muscles α-bungarotoxin activity and immunostaining for acetylcholinesterase are already recognized before E10, but currently there is no marker available which would separate presumptive intrafusal myotubes from extrafusal myotubes. As in mammals, afferent innervation is

the earliest reliable sign of a spindle anlage, and all available evidence points to a later arrival of efferents. Even diffuse acetylcholinesterase staining is not recognized at polar regions of intrafusal myotubes or fibers until E15-16 (Maier and Mayne, 1995). Localized acetylcholinesterase activity at intrafusal fiber polar regions has been noted by Mumenthaler and Engel (1961) in chicken paravertebral muscles at E18, the time when well-formed plate endings are known to occur in spindles of leg muscles (Tello, 1922). By the time of hatching motor endings are covered by Schwann cell processes and motor terminals contain many vesicles. Presynaptic and postsynaptic membranes of the neuromuscular junction are now separated by a clearly visible basal lamina (Toutant *et al.*, 1981). No authoritative descriptions exist on whether innervating motor axons are of the fusimotor or skeletofusimotor kind, or both, but Toutant *et al.* (1981) have suggested that the thin axons that establish connections at the poles are fusimotor.

Genesis of intrafusal fibers and development of MHC profiles

In chicken leg muscles primary myotubes are recognized by E5-7 (Tello, 1922; Sweeney *et al.*, 1989). It is tacitly assumed that at this age all myotubes are of the extrafusal lineage. Clearly defined, large-sized intrafusal myotubes are first seen at E13 and E14 in leg muscles (Rebollo and DeAnda, 1967; Maier, 1993a,b) and in the anterior (Toutant, 1982; Grove and Thornell, 1988) and posterior (Toutant *et al.*, 1981) latissimus dorsi muscles in sections stained for myosin ATPase or immunostained for MHC. As muscles mature, myofibrils become more densely packed in intrafusal fibers than in extrafusal fibers of the same age (Toutant *et al.*, 1981).

Early in chicken spindle development two lineages of intrafusal fibers, slow and fast, are already recognized. The contractile protein profile of the first intrafusal myotube is in most instances more fast than slow (Toutant *et al.*, 1981; Maier, 1993a), and, presumably, fast-profile primary myotubes will develop into fast intrafusal fibers of the U type (Maier and Zak, 1990; Maier, 1993a). In E15 chicken anterior latissimus dorsi muscles all intrafusal myotubes immunostain for myomesin and almost 90% of the population for M band protein. In addition, all intrafusal fibers stain for alkali-stable (fast) myosin ATPase and react with an antibody against fast MHC, while only some additional fibers stain for acid-stable (slow) myosin ATPase and coexpress slow MHC (Grove and Thornell, 1988). More often than not, the secondary chicken intrafusal myotube is slow. Fiber type composition in developing chicken spindles containing three or four intrafusal fibers suggests that the third and fourth intrafusal myotube to form are also mostly fast and slow, respectively. (Maier, 1993a). If so, spindles with three fibers would be expected to contain two fast and one slow, and spindles with four fibers two fast and two slow. In 75% or more of 8-week-old spindles actual fiber type counts are in good agreement with these predicted values (Maier, 1995b). One possible explanation for the 25% deviation is that MHC transformation occurred, as has been reported for mammals (Kucera and Walro, 1990a,b). Chicken intrafusal fibers destined to become slow transiently express ventricular myosin, but little embryonic and neonatal MHC (Maier, 1993a,b). On the other hand, presumptive fast (U) fibers never acquire ventricular myosin, but react strongly for embryonic and neonatal MHC (Maier, 1993a,b). As judged from these collective findings, the succession of avian intrafusal fiber types is a complex process (Fig. 4). Along with early type-specific MHC isoforms comes histochemical differentiation. Based on staining for oxidative enzymes, two or three types of intrafusal fiber are recognizable in chicken gastrocnemius spindles by E17 (DeAnda and Rebollo, 1968).

Initially not all young intrafusal myotubes have their own basal laminas. Instead, two or more myotubes are surrounded by a fenestrated basal lamina network. In chicken leg muscles at E16 intrafusal basal laminas are still incomplete both in terms of structural integrity and chemical composition. The specialized portion of the basal lamina that overlies the myosensory junction at the equator does not display its characteristic reactivity for chondroitin sulfate proteoglycan (Maier and Mayne, 1993) and the ß2 laminin chain (Maier and Mayne, 1995) until about E17. Intrafusal basal laminas do not become fully chemically mature until the first postnatal week.

At no time during development could typical nuclear bags be identified by Toutant *et al.* (1981) or Maier (1993a). Nuclear bags are mentioned in some earlier publications (Mumenthaler and Engel, 1960; Rebollo and DeAnda, 1967); however, these authors may have been influenced in their interpretation of structures by mammalian nomenclature, assuming that nuclear bags and nuclear chains are basic properties of all vertebrate spindles. Nuclei of sensory satellites that abut intrafusal fibers at the equator can appear under the light microscope as integral parts of intrafusal fibers.

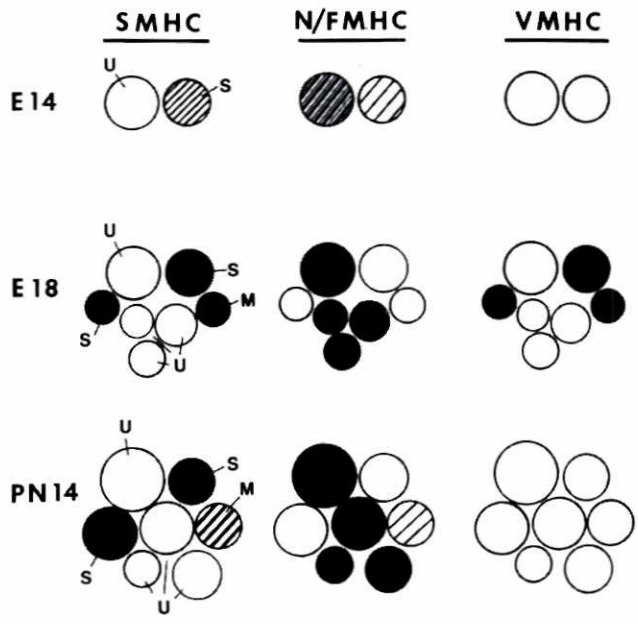

Fig. 4. Schematic representation of developing MHC profile as seen in cross-sectioned intrafusal fibers of chicken leg muscle spindles in reference to three MHC isoforms: *slow-twitch (SMHC), neonatal/fast (N/FMHC), and ventricular (VMHC) (Maier, 1992a,b, 1993a,b). The size of a circle represents the relative size of a fiber. The average number of intrafusal fibers/spindle in postnatal chicken leg muscles is about seven. Fibers are divided into two basic groups, slow and fast. The slow group has S and M subtypes, the fast group consists only of U fibers. The S and M fibers react strongly or moderately with anti-slow-twitch MHC antibodies, whereas the U fibers are unreactive (Maier and Zak, 1990). Differences in reactivity are indicated from negative (empty circle) to moderate (cross-hatched circle) to strong (black circle). The full complement of intrafusal fibers in chicken spindles is obtained by E17-18, about three to four days before hatching.*

Chicken intrafusal myogenesis continues for about 1-2 days longer than extrafusal myogenesis (Maier, 1993a). After the primary intrafusal myotube has become established, myoblasts and young myotubes are clustered around it, suggesting that new fibers align on existing ones, similar to the process that takes place in mammalian extrafusal tissue (Duxson *et al.*, 1989) and muscle spindles (Milburn, 1973, 1984; Kozeka and Ontell, 1981; Kucera *et al.*, 1988a). The greatest increase in the number of chicken intrafusal fibers takes place between E14 and E17. At the latter date virtually all intrafusal fibers have been formed (Grove and Thornell, 1988; Maier, 1993a).

Outer and inner capsules

Only a few accounts exist on capsule development. In chicken gastrocnemius an outer capsule already surrounds intrafusal fibers at E14, laid down presumably from cells of mesenchymal origin (Rebollo and DeAnda, 1967). Collagen type VI is present in the capsule at E13-14 (Maier and Mayne, 1995), and the capsular envelope is positive for lipids by E17 (DeAnda and Rebollo, 1968). In chicken posterior latissimus dorsi muscles intrafusal fibers are surrounded by a single layer of epithelial cells at E15. Extensions of these cells become rearranged to form a multilayered structure (Toutant *et al.*, 1981). As determined immunohistochemically, components of basal lamina such as laminin, collagen type IV and heparan sulfate proteoglycan are not deposited in the outer capsule until after intrafusal fiber basal laminas are fully formed. In chicken pretibial muscles, capsular basal lamina molecules are first detected at E17-18, three to four days before hatching (Maier and Mayne, 1995). By E17 there is a nascent inner capsule that surrounds intrafusal fibers only incompletely. Inner capsule cells are flat, rich in endoplasmic reticulum, and deficient in basal lamina (Toutant *et al.*, 1981). In contrast to rat and cat muscles, the periaxial space in chicken spindles is already recognizable in the late prenatal period (Toutant *et al.*, 1981).

Regeneration

As in mammals, interfering with the nerve supply to the receptor leads to structural defects. In denervated pigeon extensor digitorum communis the outer spindle capsule becomes fragmented and thickened by the addition of layers of collagen. Intrafusal fibers may hypertrophy rather than atrophy and acquire a high density of myofilaments at the equator. The collagenous perifibril sheath is unaffected (Miller and Hikida, 1986). Denervation combined with ischemia during free grafting results in more severe changes, including large gaps in the outer spindle capsule. Degenerating intrafusal fibers typically lack a sarcolemma and distinct myofibrils, and often show a disrupted basal lamina (Hikida *et al.*, 1984). The capsular and basal lamina defects seen in degenerating avian spindles may preclude regeneration of intrafusal fibers from myosatellite cells within these spindles, and in birds, in contrast to mammals (Rogers and Carlson, 1981; Rogers, 1982), spindles in regenerating muscles may arise de novo (Mackenson-Dean *et al.*, 1981). It also has been shown that spindles in regenerating bird muscles may arise *de novo*, or from remnants of pre-existing spindles (Walro *et al.*, 1984).

Cross-transplantation has been employed to explore the capacity of muscle spindle afferents to induce muscle spindles. Pigeon muscles containing spindles transplanted into the beds of muscles without spindles, allowed to degenerate and regenerate, and reinnervated by the nerves to the muscles without spindles, will not regenerate spindles. Conversely, the reciprocal setup will produce spindles in muscles normally devoid of spindles (Mackenson-Dean *et al.*, 1981). Thus, specific spindle afferents, and not just any afferent, may be required to induce muscle spindle formation.

Comment

Muscle spindles assemble from inside out, and from the equator towards the poles. Intrafusal myotubes and the afferents that

TABLE 3

SOME OBSERVATIONS IN MAMMALIAN MUSCLE SPINDLES DURING EARLY REGENERATION AFTER NEONATAL DEFICITS IN DENERVATION[1]

	Nerve Crush	*Nerve section*	*Deafferentation*	*Deefferentation*
Number of spindles	few or supernumerary[2]	none or few[3]	fewer than normally	supernumerary[2]
Number of intrafusal fibers/spindle	few	supernumerary	fewer than normally	supernumerary
Periaxial space	—	—	none or small	—
Slow-tonic MHC	—	—	not expressed	expressed, some changes in distribution
Fast-twitch MHC in intrafusal fibers	—	—	not expressed	expressed, some changes in distribution
Histochemical profile of intrafusal fibers	—	atypical	atypical	—
Sensory endings	short	few or no spirals	few or no spirals	—
Maximum firing rates of afferents	decreased	—	—	—
Static discharge during ramp-and-hold stretch	not sustained	—	—	—

[1]Recovery periods of varying lengths; [2]if combined with administration of nerve growth factor; [3]uninnervated spindles may be present .

innervate them are the first visible parts of the spindle anlage. After contact with sensory axons, intrafusal fibers grow towards the poles where their striated regions are innervated by motor axons. Intrafusal fiber basal laminas begin as an interconnecting patchwork that gives rise to fully independent basal lamina tubes after sensory and motor junction have become established. In the morphogenesis of mammalian and avian spindles the outer capsule is the last structural component to be completed. The mature outer capsule is a complex unit, consisting of perineurium and cellular and acellular components of connective tissue, both of which contribute to the make-up of the capsule from the beginning (Milburn, 1984; Maier and Mayne, 1995).

The morphological and physiological changes that occur after experimental manipulation of innervating axons are complex and not easily interpreted. In part this is due to the variety of protocols that are employed. On the whole, the concept that actions of afferents initiate spindle formation has been strengthened by data derived from degeneration/regeneration experiments. Recent work also indicates that target tissues such as muscle spindle anlagen, provide positional cues for advancing growth cones. Although during normal development Ia afferents become associated with muscle spindles and Ib afferents with tendon organs, in reinnervating cat leg muscles Ia, Ib and group II afferents may become associated with either muscle spindles or tendon organs (Banks and Barker, 1989). These actions suggest that, although powerful, afferents are not the sole inducing agents. Some basic features of regenerating spindles are listed in Table 3.

During development of avian and mammalian intrafusal fibers there is, as in extrafusal fibers, a succession of isoforms of MHC that leads to the adult profile. Primary afferents exert strong regulatory influences on the expression of MHC isoforms in mammalian intrafusal fibers; however, not all MHC profiles in normal and experimental muscles can be solely accounted for by afferent induction. Indications are that, in addition to the dominant influence of afferents, inherent properties of lines of myoblasts and motor innervation also play roles in regulating MHC expression. No data on MHC regulation via afferents or other factors are available for birds.

The first-formed mammalian nuclear bag_2 fiber is faster contracting than the later appearing nuclear bag_1 fiber (Bessou and Pagés, 1975; Boyd, 1976), properties which also may be deduced from their MHC profiles (Kucera and Walro, 1990b; Pedrosa and Thornell, 1990). Based on their MHC content (Maier, 1993a,b) and myosin ATPase (Toutant *et al.,* 1981) attributes, most chicken primary and secondary intrafusal myotubes should be fast and slow contracting, respectively. This fast-slow sequence for the first and second intrafusal myotubes is somewhat surprising because, in birds as in mammals, spindles are almost exclusively located in muscle regions that contain slow extrafusal fibers. Primary extrafusal myotubes in these areas exhibit a slow MHC profile and secondary extrafusal myotubes one that is fast (Harris *et al.,* 1989; Sweeney *et al.,* 1989; Maier *et al.,* 1991). The apparent reversal in the order of appearance between chicken intrafusal and extrafusal tissue poses the question whether primary intrafusal myotubes in terms of the original myotube pool are primary or secondary myotubes. It cannot be ruled out that initially chicken primary intrafusal myotubes are slow, and that transformation to fast MHC took place before E13, the date when they are first recognized. If there was no transformation, then a striking difference exists between rodents and cats on one hand and chickens on the other, because in chickens the fastest intrafusal fiber (U type) typically appears first, whereas in rats the fastest fibers (nuclear chain types) appear last (see Boyd, 1976). In postnatal spindles of mammalian and avian muscles both slow and fast intrafusal fibers exist; yet, the sequence of events leading to their formation apparently differs between mammals and birds.

Slow-tonic MHC is absent from extrafusal fibers of mammalian somatic muscles, but it appears in nuclear bag fibers after afferent innervation, supporting the concept that afferents induce this isoform (Kucera and Walro, 1991b). The absence of slow-tonic MHC from nuclear chain fibers may be due in part to a lesser receptivity of chain fibers to afferent induction or a degradation of afferent inductive powers over time, or both. In chickens the concept of afferent induction of specific MHC is less applicable because slow-tonic MHC occurs in populations of extrafusal and intrafusal fibers of somatic muscles (Maier, 1993a). Moreover, the intensity of immunostaining for at least one slow myosin isoform decreases in a subtype of intrafusal fibers towards the equator (Maier, 1994), directly opposite the distribution of slow-tonic MHC in rats (Kucera and Walro, 1989a). The level of afferent influence in birds is further obscured in that chicken intrafusal fibers lack nuclear bags (e.g. Maier and Eldred, 1974; Hikida, 1985), a feature thought to be induced by sensory axons.

To date no experimental data are available from birds, but excluding MHC expression, afferents in avian muscles probably also play a greater role in the induction of the spindle than do motor axons. The reason for this assertion is that sensory axons and presumptive intrafusal myotubes are recognized together at the nascent spindle, and arrival of sensory axons at the equator precedes the presence of acetylcholinesterase reactivity and well-formed neuromuscular junctions at the poles. It also can be argued that the interposition of a basal lamina between pre-and postsynaptic cells at neuromuscular junctions of the polar regions, and its absence at points of contact between sensory terminals and intrafusal fibers at the equator (Hikida, 1985; Maier and Mayne, 1995), are indications of differences in arrival of sensory and motor axons. Late establishment of fusimotor terminals at the pole finds a finished basal lamina, whereas early afferents abut directly against intrafusal fibers because basal laminas have not been laid down yet. An alternate explanation for no basal lamina at sensory terminal-intrafusal fiber contacts is the dissolution of already formed basal lamina by contacting afferents (Kucera *et al.,* 1989). Trophic molecules are a factor because neurotrophin-3-deficient mice lack basal laminas at developing neuromuscular junctions (Kucera *et al.,* 1995a), and thus, resemble mature myosensory junctions.

Neonatal deafferentation will cause in spindles that are allowed to degenerate and regenerate deficiencies in the number of intrafusal fibers and in the shape and number of sensory endings. Despite such anomalies afferent signals can be recorded, which, with the exception of maximum firing rates and sustained discharge in response to ramp-and-hold stretch, are not too atypical (Hyde and Scott, 1983; Scott and Panesar, 1995). This indicates a certain receptor plasticity with no absolute requirement for specifically structured elements, questioning the validity of detailed descriptions of sensory endings. The physiological behavior of regenerating spindles resembles that of immature postnatal spindles, in which incomplete development of afferent spirals does not prevent the issuance of a signal in response to muscle lengthening.

Most rodent (Maier *et al.*, 1974; Kucera *et al.*, 1988a; Kucera and Walro, 1990a) and cat (see Barker, 1974) spindles routinely have intrafusal fiber bundles with four, and six to seven fibers, respectively; however, in birds there is a great variation in the number of intrafusal fibers per spindle (Maier, 1983), and receptors with low intrafusal fiber counts are common (Maier and Eldred, 1974). The reason for the inconstant arrangement in birds may be one of timing. Acetylcholinesterase activity is noted in developing muscle long before the anlage of the spindle becomes visible at E13, a time when the myogenic period is declining (Tello, 1922; Maier, 1993a; Maier and Mayne, 1995). It is likely that most myotubes are already occupied by motor terminals from alpha axons, making them refractory to sensory influences. If acquisitions by alpha axons are random processes, variable numbers of myoblasts should be available for intrafusal induction, resulting in spindles with just one or two, or larger number of intrafusal fibers. While such a process might account for differences in intrafusal fiber content, it cannot explain why regions of muscles that lack slow extrafusal fibers often have few or no muscle spindles (Botterman *et al.*, 1978; Maier, 1981). One might contend that the cause for the absence of multifiber spindles in fast extrafusal fascicles is that chicken spindles virtually always contain at least one slow intrafusal fiber, and slow myotubes are absent from, or rare, in sectors where fast extrafusal fibers predominate. Even so, this still leaves unanswered why the otherwise common monofibril spindles in which the sole fiber is almost always fast are also usually absent. This paradox would seem to suggest that neuronal induction is not the only determining factor in initiating spindle development. Mutual induction between adjacent tissues is common during organogenesis (e.g. Wada *et al.*, 1996). Until recently the concept of developmental interactions as it applies to intrafusal myotubes/sensory axons has been largely biased in favor of neuronal, especially afferent regulation. One notable and welcome current trend is to explore in greater depth the area of mutual induction of neural and peripheral non-neural tissues, and the interactions of growth factors. This approach is a worthwhile undertaking in the quest towards a better understanding of muscle spindle development, and of muscle development as a whole.

Summary

The morphological and physiological development of avian and mammalian muscle spindles is reviewed, with emphasis on the recent literature. Subjects covered include the effect of sensory innervation and growth factors on the induction of muscle spindles, genesis of intrafusal fiber types as defined by isoforms of myosin heavy chains, and the establishment of the outer spindle capsule. Because of its relevance to normal development, degeneration and regeneration are also treated. Similarities and differences between mammalian and avian muscle spindle formation are discussed.

KEY WORDS: *muscle spindles, development, regeneration, morphology, physiology*

References

BANKS, R.W. and BARKER, D. (1989). Specificities of afferents reinnervating cat muscle spindles after nerve section. *J. Physiol. 408:* 345-372.

BARKER, D. (1974). The morphology of muscle receptors. In *Handbook of Sensory Physiology, II/2 Muscle Receptors* (Ed. C.C. Hunt). Springer, Berlin, pp. 1-190.

BARKER D. and IP, M.C. (1966). Sprouting and degeneration of mammalian motor axons in normal and de-afferentated skeletal muscle. *Proc. Roy. Soc. B 163:* 528-554.

BARKER, D. and MILBURN, A. (1984). Development and regeneration of mammalian muscle spindles. *Sci. Prog. Oxf. 69:* 45-64.

BARKER, D. and SCOTT, J.J.A. (1990). Regeneration and recovery of cat muscle spindles after devascularization. *J. Physiol. 424:* 27-39.

BARKER, D., SCOTT, J.J.A. and STACEY, M.J. (1985). Sensory reinnnervation of cat peroneus brevis muscle spindles after nerve crush. *Brain Res. 333:* 131-138.

BARKER, D., SCOTT, J.J.A. and STACEY, M.J. (1986). Reinnervation and recovery of cat muscle receptors after long-term denervation. *Exp. Neurol. 94:* 184-202.

BARKER, D., SCOTT, J.J.A. and STACEY, M.J. (1995). Reinnervation of cat skeleto-fusimotor units after nerve section. In *Alpha and Gamma Motor Systems* (Eds. A. Taylor, M.H. Gladden and R. Durbaba). Plenum Press, New York, pp. 196-203.

BESSOU, P. and PAGÉS, B. (1975). Cinematographic analysis of contractile events produced in intrafusal muscle fibres by stimulation of static and dynamic fusimotor axons. *J. Physiol. 252:* 397-427.

BOTTERMAN, B.R., BINDER, M.D, and STUART, D.G. (1978). Functional anatomy of the association between motor units and muscle receptors. *Am. Zool. 8:* 135-152.

BOYD, I.A. (1976). The response of fast and slow nuclear bag fibres and nuclear chain fibres in isolated cat muscle spindles to fusimotor stimulation, and the effect of intrafusal contraction on the sensory endings. *Q. J. Exp. Physiol . 61:* 203- 254.

BRAVO-REY, M.C., YAMASAKI, J.N, ELDRED, E. and MAIER, A. (1969). Ionizing irradiation on development of the muscle spindle. *Exp. Neurol. 25:* 595-602.

BUTLER, R. (1979). A new grouping of intrafusal fibers based on developmental studies of muscle spindles in the cat. *Am. J. Anat. 156:* 115-120.

COPRAY, J.C.V.M. and BROUWER, N. (1994). Selective expression of neurotrophin-3 messenger RNA in muscle spindles of the rat. *Neuroscience 63:* 1125-1135.

CUAJUNCO, F. (1927). Embryology of the neuromuscular spindle. *Contrib. Embryol. Carnegie Inst. 19:* 45-72.

CUAJUNCO, F. (1940). Development of the neuromuscular spindle in human fetuses. *Contrib. Embryol. Carnegie Inst. 28:* 95-128.

CUI, F. and WALRO, J.M. (1989). The re-expression of two myosin heavy chains in regenerated rat muscle spindles. *Neurosci. Lett. 97:* 75-79.

DEANDA, G. and REBOLLO, M.A. (1968). Histochemistry of the neuromuscular spindles in the chicken during development. *Acta Histochem. 31:* 287-295.

DIELER, R. and SCHRÖDER, J.M. (1990a). Abnormal sensory and motor reinnervation of rat muscle spindles following nerve transection and suture. *Acta Neuropathol. 80:* 163-174.

DIELER, R. and SCHRÖDER, J.M. (1990b). Increase of elastic fibres in muscle spindles of rat following single or repeated denervation with or without reinnervation. *Virchows Arch. Pathol. Anat. 417:* 213-221.

DIELER, R., VOLKER, A. and SCHRÖDER, J.M. (1992). Scanning electron microscope study of denervated and reinnervated intrafusal muscle fibers in rats. *Muscle Nerve 15:* 433-441.

DIWAN, F.H. and MILBURN, A. (1986). The effect of temporary ischemia on rat muscle spindles. *J. Embryol. Exp. Morphol. 92:* 223-254.

DOW, P.R., SHINN, S.L. and OVALLE, W.K. (1980). Ultrastructural study of a blood-muscle spindle barrier after systemic administration of horseradish peroxidase. *Am. J. Anat. 157:* 375-388.

DUXSON, M.J., USSON, Y. and HARRIS, A.J. (1989). The origin of secondary myotubes in mammalian skeletal muscles: ultrastructural studies. *Development 107:* 743-750.

ELDRED, E., YELLIN, H., DESANTIS, M. and SMITH, C.M. (1977). Supplement to bibliography on muscle receptors: their morphology, pathology physiology and pharmacology. *Exp. Neurol. 55:* 1-118.

ELDRED, E., YELLIN, H., GADBOIS, L. and SWEENEY, S. (1967). Bibliography on muscle receptors; their morphology, pathology and physiology. *Exp. Neurol. 3 (Suppl.):* 1-154.

ELLIOT, B.J. and HARRIMAN, D.G.F. (1974). Growth of human muscle spindles *in vitro*. *Nature 251:* 622-624.

ERNFORS, P., KUO-FEN, L., KUCERA, J. and JAENISCH, R. (1994). Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents. *Cell 77:* 503-512.

GLADDEN, M.H. and MILBURN, A. (1988). The motor innervation of newborn kitten muscle spindles. In *Mechanoreceptors. Development, Structure and Function* (Eds. P. Hnik, R. Vejsada and J. Zelena). Plenum Press, New York, pp. 57- 61.

GREGORY, J.E. and PROSKE, U. (1985). Responses of muscle receptors in the kitten. *J. Physiol. 366:* 27-45.

GREGORY, J.E. and PROSKE, U. (1986). Fusimotor axons in the kitten. *J. Neurophysiol. 58:* 1462-1473.

GREGORY , J.E. and PROSKE, U. (1987). Responses of muscle receptors in the kitten to succinylcholine. *Exp. Brain Res. 66:* 167-164.

GREGORY, J.E. and PROSKE, U. (1988). The response of muscle spindles in the kitten to stretch and vibration. *Exp. Brain Res. 73:* 606-614.

GREGORY, J.E. and PROSKE, U. (1991). Extrafusal and intrafusal motor units in the kitten. *Int. J. Dev. Neurosci. 9:* 101-109.

GROVE, B.K. and THORNELL, L.E. (1988). Noncoordinate expression of M band proteins in slow and fast embryonic chick muscles. *Muscle Nerve 11:* 645- 653.

HARRIS, A.J., FITZSIMONS, R.B. and MCEWAN, J.C. (1989). Neural control of the sequence of expression of myosin heavy chain isoforms in foetal mammalian muscles. *Development 107:* 751-769.

HARTUNG, V. and ASMUSSEN, G. (1988a). Der Einfluss des Lebensalters auf die extra- and intrafusalen Muskelfasern im M. soleus der Ratte. *Z. Mikrosk. Anat. Forsch. 102:* 677-693.

HARTUNG, V. and ASMUSSEN, G. (1988b). Der Einfluss des Lebensalters auf die Anzahl, Verteilung und Länge der Muskelspindeln und ihrer Kapseln im M. soleus der Ratte. *Z. Mikrosk. Anat. Forsch. 102:* 655-676.

HIKIDA, R.S. (1985). Spaced serial section analysis of the avian muscle spindle. *Anat. Rec. 212:* 255-267.

HIKIDA, R.S., WALRO, J.M. and MILLER, T.W. (1984). Ischemic degeneration of the avian muscle spindle. *Acta Neuropathol. 63:* 3-12.

HNIK, P. and ZELENA, J. (1961). Atypical spindles in reinnervated rat muscles. *J. Embryol. Exp. Morphol. 9:* 456-467.

HNIK, P., SOUKUP, T., ARUTYUNYAN, R. and UJEC, E. (1977). Responses to stretch of proprioceptors in adult rat muscles de-efferented at birth. *Pflugers Arch. 368:* 129-133.

HYDE, D. and SCOTT, J.J.A. (1983). Responses of cat peroneus brevis muscle spindle afferents during recovery from nerve crush injury. *J. Neurophysiol. 50:* 344-357.

IP, M.C. and VRBOVA, G. (1973). Motor and sensory reinnervation of fast and slow mammalian muscles. *Z. Zellforsch. Mikrosk. Anat. 146:* 261-279.

IP, M.C., LUFF , A.R. and PROSKE, U. (1988). Innervation of muscle receptors in the cross-reinnervated soleus muscle of the cat. *Anat. Rec. 220:* 212-218.

JAMES, N.T. and MEEK, G.A. (1973). An electron microscopical study of avian muscle spindles. *J. Ultrastruct. Res. 43*: 193-204.

JAMI, L., VEJSADA, R. and ZYTNICKI, D. (1989). Observations on static and dynamic responses of muscle stretch receptors in kittens. *Brain Res. 478:* 34-40.

JOZSA, L., KVIST, M., KANNUS, P. and JARVINEN, M. (1988). The effect of tenotomy and immobilization on muscle spindles and tendon organs of the rat calf muscles. *Acta Neuropathol. 76:* 465-470.

KLEIN, R., SILOS-SANTIAGO, I., SMEYNE, J., LIRA, S.A., BRAMBILLA, R., BRYANT, S., ZHANG, L., SNIDER, W.D. and BARBACID, M. (1994). Disruption of the neurotrophin-3 receptor gene *trkC* eliminates Ia muscle afferents and results in abnormal movements. *Nature 368*: 249-251.

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

KOZEKA, K. and ONTELL, M. (1981). The three-dimensional architecture of developing murine muscle spindles. *Dev. Biol. 87:* 133-147.

KUCERA, J. (1980). Myofibrillar ATPase activity of intrafusal fibers in chronically deafferented rat muscle spindles. *Histochemistry 66:* 221-228.

KUCERA, J. and WALRO, J.M. (1987). Postnatal maturation of spindles in deafferented rat soleus muscles. *Anat. Embryol. 176:* 449-461.

KUCERA, J. and WALRO, J.M. (1988). The effect of neonatal deafferentation on myosin heavy chain expression in intrafusal muscle fibers of the rat. *Histochemistry 90:* 151-160.

KUCERA, J. and WALRO, J.M. (1989a). Nonuniform expression of myosin heavy chain isoforms along the length of cat intrafusal muscle fibers. *Histochemistry 92*: 291-299.

KUCERA, J. and WALRO, J.M. (1989b). Postnatal expression of myosin heavy chains in muscle spindles of the rat. *Anat. Embryol. 179:* 369-376.

KUCERA, J. and WALRO, J.M. (1989c). Alteration in intrafusal fiber composition by nerve crush in neonatal rats. *Neurosci. Lett. 105:* 552-556.

KUCERA, J. and WALRO, J.M. (1990a). Origin of intrafusal muscle fibers in the rat. *Histochemistry 93:* 567-580.

KUCERA, J. and WALRO, J.M. (1990b). Myosin heavy chain expression in developing rat intrafusal muscle fibers. *Neurosci. Lett. 109:* 18-22.

KUCERA, J. and WALRO, J.M. (1990c). Treatment with beta bungarotoxin blocks muscle spindle formation in fetal rats. *Development 110:* 483-489.

KUCERA, J. and WALRO, J.M. (1991a). Slow-tonic MHC expression in paralyzed hindlimbs of fetal rats. *Histochemistry 96:* 51-57.

KUCERA, J. and WALRO, J.M. (1991b). Aggregation of myonuclei and the spread of slow-tonic myosin immunoreactivity in developing muscle spindles. *Histochemistry 96:* 381-390.

KUCERA, J. and WALRO, J.M. (1992a). Superfluousness of motor innervation for the formation of muscle spindles in neonatal rats. *Anat. Embryol. 186:* 301-309.

KUCERA, J. and WALRO, J.M. (1992b). Formation of muscle spindles in the absence of motor innervation. *Neurosci. Lett. 145:* 47-50.

KUCERA, J. and WALRO, J.M. (1994). Sequences of intrafusal fiber formation are muscle-dependent in rat hindlimbs. *Anat. Embryol. 190:* 273-286.

KUCERA, J. and WALRO, J.M. (1995). Origin of intrafusal fibers from a subset of primary myotubes in the rat. *Anat. Embryol. 192:* 149-158.

KUCERA, J., ERNFORS, P., SCHWARZE, J. and JAENISCH, R. (1995a). Early development of the muscle spindle and its dependence on neurotrophic factors. In *Alpha and Gamma Motor Systems* (Eds. A. Taylor, M. H. Gladden and R. Durbaba). Plenum Press, New York, pp. 183-188.

KUCERA, P., ERNFORS, P., WALRO, J.M. and JAENISCH, R. (1995b). Reduction in the number of spinal motor neurons in neurotrophin-3-deficient mice. *Neuroscience 69:* 321-330.

KUCERA. J., WALRO, J.M. and REICHLER, J. (1988a). Innervation of developing intrafusal muscle fibers in the rat. *Am. J. Anat. 183:* 344-358.

KUCERA, J., WALRO, J.M. and REICHLER, J. (1988b). Motor and sensory innervation of muscle spindles in the neonatal rat. *Anat . Embryol. 177:* 427-35.

KUCERA, J., WALRO, J.M. and REICHLER, J. (1989). Role of nerve and muscle factors in the development of rat muscle spindles. *Am. J. Anat. 186:* 144-160.

KUCERA, J., WALRO, J.M. and GAO, Y. (1993a). Fusimotor-free spindles in reinnervated muscles of neonatal rats treated with nerve growth factor. *Neuroscience 52:* 219-228.

KUCERA, J., WALRO, J.M. and GAO, Y. (1993b). Influence of muscle cell substrates on differentiation of intrafusal fiber types in neonatal rats. *Neuroscience 52:* 1001-1008.

KUCERA, J., WALRO, J.M. and GORZA, L. (1992). Expression of type-specific MHC isoforms in rat intrafusal muscle fibers. *J. Histochem. Cytochem. 40*: 293-307.

LANDON, D.N. (1972). The fine structure of the equatorial region of developing muscle spindles in the rat. *J. Neurocytol. 1:* 189-210.

MACKENSON-DEAN, C.A., HIKIDA, R.S. and FRANGOWLAKIS, T.M. (1981). Formation of muscle spindles in regenerated avian muscle grafts. *Cell Tissue Res. 217:* 37-41.

MAEDA, N., OSAWA, K., MASUDA, T., HAKEDA, Y. and KUMEGAWA, M. (1985). Postnatal development of the anulospiral endings of Ia fibers in muscle spindles of mice. *Acta Anat. 124:* 42-46.

MAEDA, N., KAWASAKI, T., OSAWA, K., SUMIDA, H., YAMAMOTO, Y., MASUDA, T., SUWA T., WATANABE, Y. and KUMEGAWA, M. (1988a). Effects of fine grained-diet on anulospiral endings of muscle spindles in the masseter muscle in developing and adult mice. *Anat . Anz. 165:* 177-192.

MAEDA, N., SATO, M., OSAWA, K., KAWASAKI, T., MASUDA, T. and KUMEGAWA, M. (1988b). Postnatal development of the mouse temporal muscle and effects of fine-grained diet on the muscle spindle. *Anat. Anz. 165:* 177-192.

MAEDA, N., OSAWA, K. , MASUDA, T., SUWA, T. and KUMEGAWA, M. (1989). Prolonged degeneration of muscle spindles in the masseter muscle after treatment of developing mice with the local anesthetic lidocaine hydrochloride. *Acta Anat. 135:* 318-322.

MAIER, A. (1981). Characteristics of pigeon gastrocnemius and its muscle spindle supply. *Exp. Neurol. 74:* 892-906.

MAIER, A. (1983). Differences in muscle spindle structure between pigeon muscles used in aerial and terrestrial locomotion. *Am. J. Anat. 168:* 27-36.

MAIER, A. (1989). Contours and distribution of sites that react with antiacetylcholinesterase in chicken intrafusal fibers. *Am. J. Anat. 185:* 33-41.

MAIER, A . (1992a). The avian muscle spindle. *Anat. Embryol. 186:* 1-25.

MAIER, A. (1992b). Fast and slow intrafusal fibre systems in chicken leg muscle spindles. *J. Anat. 180:* 233-237.

MAIER, A. (1993a). Development of chicken intrafusal muscle fibers. *Cell Tissue Res.* 274: 383-391.

MAIER, A. (1993b). Transient expression of a ventricular myosin heavy chain isoform in developing chicken intrafusal muscle fibers. *Histochemistry 99:* 333-338.

MAIER, A. (1994). Type and regional diversity in the distribution of myosin heavy chains in chicken intrafusal muscle fibers. *Anat. Rec. 240:* 507-515.

MAIER. A. (1995a). Equatorial morphology of slow and fast intrafusal fibers in chicken leg muscle spindles. *Soc. Neurosci. Abstr. 21:* 1663.

MAIER, A. (1995b). Fiber-type compositions in postnatal chicken muscle spindles with low intrafusal fiber counts and their developmental significance. *J. Morphol. 224:* 171-177.

MAIER, A. and ELDRED, E. (1974). Postnatal growth of the extra- and intrafusal fibers in the soleus and medial gastrocnemius of the cat. *Am. J. Anat. 141:* 161-178.

MAIER, A. and MAYNE, R. (1993). Regional differences in organization of the extracellular matrix and cytoskeleton at the equator of chicken intrafusal muscle fibres. *J. Muscle Res. Cell Motil. 14:* 35-46.

MAIER, A. and MAYNE, R. (1995). Basal lamina development in chicken muscle spindles. *Dev. Dynamics 202:* 284-293.

MAIER, A. and ZAK, R. (1990). Presence in chicken tibialis anterior and extensor digitorum longus muscle spindles of reactive and unreactive intrafusal fibers after incubation with monoclonal antibodies against myosin heavy chains. *Am. J. Anat. 187:* 338-346.

MAIER, A., GAMBKE, B. and PETTE, D. (1988). Immunohistochemical demonstration of embryonic myosin heavy chains in adult mammalian intrafusal fibers. *Histochemistry 88:* 267-271.

MAIER, A., McEWAN, J.C., DODDS, K.G., FISHMAN, D.A., FITZSIMONS, R.B. and HARRIS, A.J. (1991). Myosin heavy chain composition of single fibres and their origins and distribution in developing fascicles of sheep tibialis cranialis muscles. *J. Muscle Res. Cell Motil. 13:* 551-572.

MAIER, A., SIMPSON, D.R. and EDGERTON, V.R. (1974). Histochemical profiles of guinea pig intrafusal fibres in normal muscles and after denervation, cordotomy and tenotomy. *Histochem. J. 6:* 665-677.

MARCHAND, R. and ELDRED, E. (1969). Postnatal increase of intrafusal fibers in the rat muscle spindle. *Exp. Neurol. 25:* 655-676.

MATSUMOTO, D.E. and BAKER, J.H. (1987). Degeneration and alteration of axons and intrafusal muscle fibers in spindles following tenotomy. *Exp. Neurol. 97:* 482-498.

MAVRINSKAYA, L.F. (1967). Development of muscle spindles in man. *Arkh. Anat. Gistol. Embryol. 53:* 42-49.

MESSERSMITH, E.K., LEONARDO, E.D., SHATZ, C.J., TESSIER-LAVIGNE, M., GOODMAN, C.S. and KOLODKIN, A.L. (1995). Semaphorin III can function as a selective chemorepellent to pattern sensory projections in the spinal cord. *Neuron 14:* 949-959.

MILBURN, A. (1973). The early development of muscle spindles in the rat. *J. Cell Sci. 12:* 175-195.

MILBURN, A. (1976). The effect of the local anesthetic bupivacaine on the muscle spindle of rat. *J. Neurocytol. 5:* 425-446.

MILBURN, A. (1984). Stages in the development of cat muscle spindles. *J. Embryol. Exp. Morphol. 82:* 177-216.

MILLER, T.W. and HIKIDA, R.S. (1986). Effects of short-term denervation on avian muscle spindle structure. *Acta. Neuropathol. 70:* 127-134.

MUMENTHALER, M. and ENGEL, W.K. (1961). Cytological localization of cholinesterase in developing chick embryo skeletal muscle. *Acta. Anat. 47:* 274-299.

NOVOTOVÁ, M. and SOUKUP, T. (1995). Neomyogenesis in neonatally de- efferented and postnatally denervated rat muscle spindles. *Acta Neuropathol. 89:* 85-95.

OAKLEY, R.A., GARNER, A.S., LARGE, T.H. and FRANK, E. (1995). Muscle sensory neurons require neurotrophin-3 from peripheral tissues during the period of normal cell death. *Development 121:* 1341-1350.

OSAWA, K., MAEDA, N., SATO, M., KAWASAKI, T., MASUDA, T., YAMAMOTO, Y., HAKEDA, Y., UKAI M., WATANABI, Y., SUWA, T. and KUMEGAWA, M. (1988). Postnatal development of the anulospiral endings of Ia fibers in muscle spindles of the mouse temporalis muscle. *Anat. Anz. 167:* 253-257.

OVALLE, W.K. (1976). Postnatal cytochemical development of muscle fibres in segmental tail muscles of the rat. *J. Embryol. Exp. Morphol. 35:* 425-435.

PALECEK, J., VEJSADA, R., SOUKUP, T. and HNIK, P. (1989). Function and structure of atypical muscle spindles after neonatal nerve crush in rats. *Exp. Brain Res. 74:* 417-420.

PATAK, A., PROSKE, U., TURNER, H. and GREGORY, J.E. (1992). Development of the sensory innervation of muscle spindles in the kitten. *Int. J. Dev. Neurosci. 10:* 81-92.

PEDROSA, F. and THORNELL, L.E. (1990). Expression of myosin heavy chain isoforms in developing rat muscle spindles. *Histochemistry 94:* 231-244.

PEDROSA, F., SOUKUP, T. and THORNELL, L.E. (1990). Expression of an alpha cardiac-like myosin heavy chain in muscle spindle fibers. *Histochemistry 95:* 105-111.

PEDROSA-DOMELLÖF, F. (1991). Expression of myosin heavy chain isoforms in rat muscle spindles. *Umea Medical Dissertations No. 304*, University of Umea, Umea.

PEDROSA-DOMELLÖF, F., SOUKUP, T. and THORNELL, L.E. (1991). Rat muscle spindle histochemistry revisited. *Histochemistry 96:* 327-338.

QUICK, D.C. and ROGERS, S.L. (1983). Stretch receptors in regenerated rat muscles. *Neuroscience 10:* 851-859.

REBOLLO, M.A. and DeANDA, G. (1967). Morphology of neuromuscular spindles in the chicken during development. *Acta. Neurol. Latinoam. 13:* 150-155.

ROGERS, S.L. (1982) Muscle spindle formation and differentiation in regenerating rat muscle grafts. *Dev. Biol. 94:* 265-283.

ROGERS, S.L. and CARLSON, B.M. (1981). A quantitative assessment of muscle spindle formation in reinnervated and non-reinnervated grafts of the rat extensor digitorum longus muscle. *Neuroscience 6:* 87-94.

SCHIAFFINO, S. and PIEROBON-BORMIOLI, S. (1976). Morphogenesis of rat muscle spindles after nerve lesion during early postnatal development. *J. Neurocytol. 5:* 319-336.

SCHOBER, R. and YAMAMURA, Y. (1983). Zur Feinstruktur intrafusaler und extrafusaler Nervenabschnitte der Rattenmuskelspindel nach Ischiadusdurch trennung. *Acta Neuropathol. 59:* 246-254.

SCHRÖDER, J.M. (1974a). The fine structure of de-and reinnervated muscle spindles. I. The increase, atrophy and "hypertrophy" of intrafusal fibers. *Acta Neuropathol. 30:* 109-128.

SCHRÖDER, J.M. (1974b). The fine structure of de- and reinnervated muscle spindles. II. Regenerated sensory and motor nerve terminals. *Acta Neuropathol. 30:* 129-144.

SCHRÖDER, J.M., KEMME, P.T. and SCHOLZ, L. (1979). The fine structure of denervated and reinnervated muscle spindles: morphometric study of intrafusal muscle fibers. *Acta Neuropathol. 46:* 95-106.

SCOTT, J.J.A. (1987). The reinnervation of cat muscle spindles by skeletofusimotor axons. *Brain Res. 401:* 152-154.

SCOTT, J.J.A. and PANESAR, G. (1995). Small-signal sensitivity of muscle spindles reinnervated after long-term denervation. *Brain Res. 671:* 325-328.

SEKIYA, S., HOMMA, S., MYATA, Y. and KUNO, M. (1986). Effects of nerve growth factor on differentiation of muscle spindles following nerve lesions in neonatal rats. *J. Neurosci. 6:* 2019-2025.

SHERRINGTON, C.S. (1894). On the anatomical constitution of nerves of skeletal muscles; with remarks on recurrent fibres in the ventral spinal nerve roots. *J. Physiol. 17:* 211-258.

SKOGLUND, S. (1960). The activity of muscle receptors in the kitten. *Acta. Physiol. Scand. 50:* 203-221.

SOUKUP, T. and ZELENA, J. (1985). The differentiation of muscle stretch receptors in the rat after neonatal de-efferentation. *Physiol. Bohemoslov. 34 (Suppl.):* 153-156.

SOUKUP, PEDROSA, F. and THORNELL, L.E. (1990). Influence of neonatal motor denervation on expression of myosin heavy chain isoforms in rat muscle spindles. *Histochemistry 94:* 245-256.

SOUKUP, T., PEDROSA-DOMELLÖF, F. and THORNELL, L.E. (1993). Differentiation of supernumerary fibres in neonatally deefferented rat muscle spindles. *Differentiation 53:* 35-43.

SOUKUP, T., PEDROSA-DOMELLÖF, F. and THORNELL, L.E. (1995). Expression of myosin heavy chain isoforms and myogenesis of intrafusal fibres in rat muscle spindles. *Microsc. Res. Tech. 30:* 390-407.

SWASH, N. and FOX, K.P. (1974). The pathology of the human muscle spindle. Effect of denervation. *J. Neurol. Sci. 22:* 1-24.

SWATLAND, H. J. (1974). Fetal and neonatal development of spindle capsules and intrafusal myofibers in the porcine sartorius. *J. Anim. Sci. 39:* 42-46.

SWEENEY, L.J., KENNEDY, J.M., ZAK, R., KOKJOHN, K. and KELLEY, S.W. (1989). Evidence for expression of a common myosin heavy chain phenotype in future fast and slow skeletal muscle embryogenesis. *Dev. Biol. 133:* 361-374.

TELLO, J.F. (1922). Die Entstehungen der motorischen und sensiblen Nervenendingungen. I. In dem lokomotorischen System der höheren Wirbeltiere. Muskuläre Histogenese. *Z. Anat. Entw.-Gesch. 6:* 348-440.

teKRONNIE, G., DONSELAAR, Y., SOUKUP, T. and ZELENA, J. (1982). Development of immunohistochemical characteristics of intrafusal fibers in normal and de-efferented rat muscle spindles. *Histochemistry 74:* 355-366.

THORNELL, L.E., GROVE, B.K., PEDROSA, F., BUTLER-BROWNE, G.S., DHOOT, G.K. and FISCHMAN, D.A. (1989). Expression of slow tonic myosin in muscle spindle fibers early in mammalian development. In *Cellular and Molecular Biology of Muscle Development* (Ed. F.E. Stockdale). Liss, New York, pp. 471-480.

TOUTANT, M. (1982). Quantitative and histochemical aspects of differentiation of muscle spindles in the anterior latissimus dorsi of the developing chick. *Anat. Embryol. 163:* 475-485.

TOUTANT, M., BORGEOIS, J.P., ROUAUD, T. and TOUTANT, J.P. (1981). Morphological and histochemical differentiation of intrafusal fibres in the posterior latissimus dorsi muscle of the developing chick. *Anat. Embryol. 162:* 325-342.

VEJSADA, R., HNIK, P., JAMI, L., PAYNE, R. and ZYTNICKI, D. (1988). Developmental aspects of stretch receptor function in the rat and the cat. In *Mechanoreceptors. Development, Structure and Function* (Eds. P. Hnik, T. Soukup, R. Vejsada and J. Zelena). Plenum Press, New York, pp. 63-70.

WADA, J., KUMAR, A., LIU, Z, RUOSLAHTI, E., REICHARDT, L., MARVALDI, J. and KANWAR, Y.S. (1996). Cloning of mouse integrin "α_v cDNA and role of the "α_v-related matrix receptors in metanephric development. *J. Cell Biol. 132:* 1161-1176.

WALRO, J.M. and WANG, J. (1995). Origin of muscle spindles in developing rat hindlimb muscles. In *Alpha and Gamma Motor Systems* (Eds. A. Taylor, M.H. Gladden and R. Durbaba). Plenum Press, New York, pp. 189-195.

WALRO, J.M., HIKIDA, R.S. and HATCHER, B.M. (1984). Quantification and origin of spindles in orthotopic and heterotopic grafts of avian muscles. *Cell Tissue Res. 235:* 515-519.

WALRO, J.M., KUCERA, J. and NARVY, R. (1991). Non-neural and neural expression of myosin heavy chains by regenerated intrafusal fibers of rats. *Neurosci. Lett. 122:* 213-217.

WALRO, J.M., KUCERA. J., CUI, F. and STAFFELD, C.G. (1989). Innervation of regenerated spindles in muscle grafts of the rat. *Histochemistry 92:* 1-13.

WEISMANN, A. (1861). Über das Wachstum der quergestreiften Muskeln nach Beobachtungen am Frosch. *Z. Ration. Med. 10:* 263-284.

WENNER, P. and FRANK, E. (1995). Peripheral target specification of synaptic connectivity of muscle spindle sensory neurons with spinal motoneurons. *J. Neurosci. 15:* 8191-8198.

WERNER, J.K. (1973a). Duration of normal innervation required for complete differentiation of muscle spindles in newborn rats. *Exp. Neurol. 41:* 214-217.

WERNER, J.K. (1973b). Mixed intra-and extrafusal muscle fibers produced by temporary denervation in newborn rats. *J. Comp. Neurol. 150:* 279-302.

WHALEN, R.G., SELL, S.M., BUTLER-BROWNE, G.S., SCHWARTZ, K., BOUVERET, P. and PINSON-HARSTROM, I. (1981). Three myosin heavy chains appear sequentially in rat muscle development. *Nature 292:* 805-809.

WIRSÉN, C. and LARSSON, K.S. (1964). Histochemical differentiation of skeletal muscle in fetal and newborn mice. *J. Embryol. Exp. Morphol. 12:* 759-767.

WOODBURY, C.F. and SCOTT, S.A. (1995). Cutaneous and muscle afferents: Interactions with potential targets *in vitro. J. Neurosci. 15:* 3936-3951.

YAMURA, Y. and SCHOBER, J.M. (1982). Zur Feinstruktur der Nervenendigungen in den Muskelspindeln des M. lumbricalis der Ratte nach Durchschneidung des Nerven. *Acta Neuropathol. 57:* 23-63.

YELLIN, H. and ELDRED, E. (1970). Spindle activity of the tenotomized gastrocnemius muscle in the cat. *Exp. Neurol. 29:* 513-533.

ZELENA, J. (1957). The morphogenetic influence of innervation on the ontogenetic development of muscle spindles. *J. Embryol. Exp. Morphol. 5:* 283-294.

ZELENA, J. (1962). The effect of denervation on muscle development. In *The Denervated Muscle* (Ed. E. Gutman). Publishing House of the Czechoslovac Academy of Sciences, Prague, pp. 103-106.

ZELENA, J. (1963). Development of muscle receptors after tenotomy. *Physiol. Bohemoslov. 12:* 30-36.

ZELENA, J. (1964). Development, degeneration and regeneration of receptor organs. In *Progress in Brain Research,* Vol. 13 (Eds. M. Singer and J.P. Schade). Elsevier, Amsterdam, pp. 175-213.

ZELENA, J. and HNIK, P. (1960). Absence of spindles in muscles of rat reinnervated during development. *Physiol. Bohemoslov. 9:* 373-381.

ZELENA, J. and SOUKUP, T. (1973). Development of muscle spindles deprived of fusimotor innervation. *Z. Zellforsch. 144:* 435-452.

ZELENA, J. and SOUKUP, T. (1974). The differentiation of intrafusal fiber types in rat muscle spindles after motor denervation. *Cell Tissue Res. 153:* 115-136.

ZELENA, J. and SOUKUP, T. (1993). Increase in the number of intrafusal muscle fibers in rat muscles after neonatal motor denervation. *Neuroscience 52:* 207-218.

ZHOU, X.F. and RUSH, R.A. (1995). Peripheral projections of rat primary sensory neurons immunoreactive for neurotrophin 3. *J. Comp. Neurol. 363:* 69-77.

Received: July 1996
Accepted for publication: November 1996