Denervation of Primate Extraocular Muscle

A Unique Pattern of Structural Alterations

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Extraocular muscles differ from most other skeletal muscles in terms of constituent fiber types and innervation pattern. The rules that govern fiber responses to various experimental interventions for most skeletal muscles, therefore, may not strictly apply to the extraocular muscles. In this study, denervation of the extraocular muscles of Cynomolgous monkeys, *Macaca fascicularis*, was accomplished by intracranial transection of the oculomotor nerve. Survival times of 3–167 days were allowed, and muscles were processed for analysis by light and electron microscopy. Short-term alterations involved all muscle fiber types and included retraction of neuromuscular junctions, mild myofibril disruption and inflammatory cell infiltration. Long-term morphopathological changes were most apparent in the orbital singly innervated fiber type and its global layer counterpart. These alterations consisted of dispersion of the mitochondrial aggregates which characterize this fiber type. Only occasional fibers (all types) exhibited severe vacuolar atrophy or myofilament breakdown despite the occurrence of only limited reinnervation. When extensive reinnervation did occur, the characteristic layered organization of the extraocular muscles was preserved and fiber type grouping was not apparent. Taken together, these findings indicate that the extraocular muscles exhibit a resilience to denervation beyond that observed for other skeletal musculature. Invest Ophthalmol Vis Sci 30:1894–1908, 1989

The extraocular muscles are unique among mammalian skeletal muscles. Histochemical and ultrastructural analyses have demonstrated that the conventional muscle fiber classification schemes are not applicable to the extraocular muscles.1 Instead, monkey extraocular muscles contain an unusual complement of six distinct fiber types, covering the spectrum from fast-twitch to slow-tonic fibers. Segregation of eye movement function among what are potentially six different motor unit types is a poorly understood issue in the oculomotor system. Indeed, studies of extraocular muscle motor unit properties2–5 are few and have not resolved more than two to three motor unit types.

Because of the differences between extraocular and other skeletal muscles, it is possible that these muscles might respond differently when subjected to experimental manipulations. Unique fiber type-specific responses have been reported for primate extraocular muscles subsequent to exposure to retrobulbar anesthetics6 or intramuscular botulinum toxin.7 However, many studies concerned with extraocular muscle pathology have failed to distinguish between generalized and fiber type-specific alterations. Recognition of fiber type-specific alterations in experimentally induced or naturally occurring pathology may provide insights into the division of labor in the extraocular muscles.8

The current study examines structural alterations in primate extraocular muscle that result from removal of motor innervation. Patterns of generalized and fiber type-specific morphopathological change are described. The extraocular muscles are unique in that denervation does not induce the profound myofilament loss that characterizes other skeletal muscle. The most conspicuous effect of denervation was dispersion of the central and peripheral mitochondrial aggregates of the orbital singly innervated muscle fiber type. Given the normal morphology (indicative of high oxidative capacity and thus, ability for prolonged contraction) and particular sensitivity of the...
orbital singly innervated fiber to denervation, it is likely that this fiber is recruited early in eye movements and is active over much of the oculomotor range. Preliminary results have appeared elsewhere.

### Materials and Methods

Unilateral intracranial transection of the oculomotor nerve was performed under aseptic conditions in young adult Cynomolgous monkeys (Macaca fascicularis) weighing between 3.5 and 4.5 kg. Following induction of anesthesia with pentobarbital sodium (35 mg/kg, IV), a left temporal craniotomy (approximately 1 cm diameter) was performed, the dura was incised, and the temporal lobe was retracted upward to expose the oculomotor nerve just behind its entrance into the cavernous sinus. Retraction of the cortex was aided by administration of intravenous furosemide and mannitol. The oculomotor nerve was torn with a blunt nerve hook such that the nerve usually was severed at its junction with the brainstem (no attempt was made to completely remove a segment of nerve). Gross tearing of the nerve effectively delayed or prevented reinnervation, thereby allowing assessment of the consequences of long-term denervation. Verification of complete nerve transection was obtained both by visual inspection and by the resulting loss of the direct pupillary light reflex. Monkeys subsequently developed permanent exodeviation, ptosis (which resolved after 28–56 days) and mydriasis (which did not resolve) of the ipsilateral eye. The surgical wound was closed in layers, and dexamethasone and prophylactic antibiotics were administered. Meperidine was given to alleviate postoperative discomfort. In an additional experimental case, however, the oculomotor nerve was cut cleanly at the point of entrance into the wall of the cavernous sinus in order to enhance the likelihood of reinnervation. All procedures involving animals conformed to the ARVO Resolution on the Use of Animals in Research.

Following survival times of 3–167 days (two animals each at 3, 7, 14, 28, 56, 112 and 167 days), monkeys were again anesthetized with pentobarbital sodium, heparinized, intubated and artificially resired with 95% oxygen/5% carbon dioxide prior to sacrifice. Monkeys were then overdosed with pentobarbital sodium and perfused transcardially with 1000 ml of physiological saline followed by 3000 ml of fixative solution containing 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Selected extraocular muscles were then removed from experimental and unoperated control orbits, postfixed for two hours in cold fixative containing 4% glutaraldehyde in 0.1 M phosphate buffer, and washed in cold phosphate buffer overnight. While other muscles were examined, these studies largely focused upon the medial rectus. Muscles were cut transversely at 500 μm using a Smith-McLwain tissue sectioner and were processed for the histochemical localization of acetylcholinesterase (AChE) using a copper thioccholine precipitation technique. Tetraisopropyl pyrophosphoramide (iso-OPMA, 10^{-4} M; Sigma Chemical Co., St. Louis, MO) was used as an inhibitor of nonspecific cholinesterase. AChE reaction product allowed localization of sites, or former sites, of neuromuscular junctions. Muscle sections were postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer, stained en bloc with 0.05% uranyl acetate in maleate buffer, dehydrated in a graded series of methanols and propylene oxide, and embedded in TAAB 812 resin. Semithin sections (1 μm) encompassing the entire cross-sectional area of the muscle were cut with an ultramicrotome, stained with 1% toluidine blue or 0.1% para-phenylenediamine, and examined with brightfield or phase contrast microscopy.

For some survival times, all muscle fibers in single semithin sections through the innervation zone (approximately 9000 to 15,000 fibers per muscle) were subjected to morphometric analysis using a Microcomp image analysis system (Southern Micro Instruments, Atlanta, GA) equipped with standard statistical software. While viewing a display screen with graphics overlay, the system operator (one of the authors) distinguished muscle fiber types and, using a graphics pad, outlined the perimeter of each fiber. The computer used an algorithm to calculate fiber cross-sectional area and stored these data on disk. In all qualitative and quantitative analyses, particular attention was paid to the evaluation of muscle fiber type-specific changes. Fiber type cross-sectional areas were expressed as a percent of the matched control value. Data obtained from the intact contralateral medial rectus of each animal served as the control for that experiment (this avoided inter-animal variables such as tissue shrinkage); again, all fibers in single cross-sections through the innervation zone of control muscles were digitized. Student t-test was used to compare experimental values at the selected survival times with their matched control values (a stringent level for significance, P < 0.001, was chosen). Since quantitative data were obtained from only one animal at each survival time, no attempts were made to apply statistical analysis across survival times.

Ultrathin sections (60–80 nm) containing representative portions of the orbital or global zone were cut with the ultramicrotome using a diamond knife,
Fig. 1. Phase contrast light photomicrographs of normal (A, D) and denervated (B, C, E, F) monkey medial rectus muscle. The orbital layer (A) contains one singly innervated fiber type (examples indicated by 1) and one multiply innervated fiber type (2). Progressive reduction of the cross-sectional area of both orbital fiber types, and loss of the distinctive granularity (indicative of mitochondrial content, m) of the singly innervated fiber is seen 28 (B) and 56 (C) days after oculomotor neurectomy. The global layer (D) contains three types of singly innervated fiber (3, 4, 5) and one multiply innervated fiber type (6). Similar reduction of singly innervated fiber cross-sectional area is seen after 28 (E) and 56 (F) days, although, with the exception of the type 3 fiber, mitochondrial changes are not as severe. Mitochondrial loss occurring by 56 days (F) precludes subdivision of the different singly innervated fiber types; thus, singly innervated fibers are indicated by asterisks (*). The global multiply innervated fiber (6) exhibits no change (×565).
Fig. 2. Phase contrast light photomicrographs of oculomotor nucleus at 56 (A) and 28 (B, C) days after oculomotor nerve transection. Low-magnification micrograph (A) illustrates severe reduction in number of large neurons, putative motoneurons, on left as compared to the control unlesioned side on right. Arrows indicate representative neurons. Motoneurons undergo a chromatolytic response (n), exhibiting peripheral nuclei and swollen cytoplasm (B) in contrast to normal (C). ×120 (A), ×480 (B, C).

picked up on formvar-coated single-slot grids, and stained with uranyl acetate and lead citrate. Sections were examined and photographed using a Zeiss 10C electron microscope.

Results

The extraocular muscles contain six distinct muscle fiber types distributed among orbital, intermediate, and global zones (Fig. 1). Significantly differences in the constituent fiber types and innervation patterns exist between the extraocular muscles and most other skeletal muscles such that conventional muscle fiber classification schemes are inapplicable, if not inappropriate. Fiber type identification criteria included: (1) innervation pattern (singly innervated versus multiply innervated); (2) number, size and distribution of mitochondria; and (3) degree of development of internal membrane systems (sarcoplasmic reticulum and t-tubule systems). This muscle fiber classification scheme has been described in detail elsewhere. The six extraocular muscle fiber types exhibited both generalized and differential responses to chronic removal of their motor innervation.

Oculomotor Motoneuron Response

Centrally placed oculomotor nerve lesions resulted in loss of most of the motoneurons in the ipsilateral oculomotor nucleus. Except for the superior rectus subdivision, which projects through the contralateral oculomotor nerve, less than 10% of putative motoneurons survived. Some surviving neurons may represent oculomotor internuclear neurons. Axotomized oculomotor motoneurons underwent a modified chromatolytic response (see also Spencer and Baker) and were eliminated by 56 days (Fig. 2). Within the first week after oculomotor nerve transection, preterminal axons and axon terminals exhibited swelling and mitochondrial aggregations irrespective of the muscle fiber type that they served. Mitochondrial cristae became swollen and disrupted, and neurofilament dispersion and terminal vacuol-
Infiltration of Immune System Elements

An infiltration by blood-borne immune system elements was noted within 56 days following oculomotor neurectomy (Fig. 4). By 14 days, evidence of breakup and phagocytosis of vacant myelin sheaths was noted, and focal perivascular sites of mononuclear cell infiltration were observed. Schwann cells contained accumulations of distinctive lysosomes, referred to as laminated cytoplasmic bodies, suggesting that they may play a role in elimination of the empty myelin (Fig. 4B). Monton et al. indicate that laminated bodies are probably autophagic vacuoles containing acid phosphatase and slowly degrading myelin-derived lipids. Similar Schwann cell lysosomes have been reported in association with myelin degeneration in the extraocular muscles of patients with Apert's syndrome. Between 28 and 56 days, a massive accumulation of monocytes, lymphocytes, mast cells, and some eosinophils and plasma cells occurred. Both granulated and degranulating mast cells were seen during this time period. Accumulation of immune system elements was not a generalized orbital response since the lateral rectus muscles (for which innervation was intact) did not exhibit such infiltrations. A shift in the composition of the cellular infiltrate, from immune system elements toward increased numbers of connective tissue fibroblasts and fat cells, occurred after 112 days.

General Muscle Alterations

Over the 167-day survival range that was examined in this study, the most obvious qualitative change, namely dispersion of muscle fiber mitochondria, was exhibited by the orbital and global singly innervated
fiber types (Fig. 1). The greatest reduction in myofiber granularity occurred between 14 and 56 days after oculomotor nerve transection. Multiply innervated fiber types did not exhibit such mitochondrial alterations. In spite of the relative absence of reinnervation, there was no gross atrophy or significant fiber loss. The total number of fibers, 10,000 to 15,000 per medial rectus muscle, was not significantly changed by denervation for the survival times examined. Morphometric analysis of the muscle as a whole (all fiber types) was consistent with this pattern of mild qualitative changes. These data showed a reduction in mean fiber cross-sectional area, with values leveling off at 75–80% of control (Fig. 5) for long post-denerv-
Fig. 5. Histogram illustrating mean fiber cross-sectional area expressed as a percentage of mean control value for each of four post-denervation survival times. A value of 100% indicates that experimental and control values were identical; fiber hypertrophy is indicated by values > 100, atrophy indicated by values < 100. Two 167-day survival cases are indicated; one of which exhibited extensive reinnervation (a) while the other showed only limited reinnervation (b). Note the progressive reduction in area, followed by hypertrophy after reinnervation, for orbital and global singly innervated fibers (SIF) and orbital multiply innervated fiber (MIF). Global MIF shows little change. Differences significant from control at the level of \( P < 0.001 \), according to student t-test, are designated (asterisks).

Muscle Fiber Type-Specific Alterations

Reminiscent of alterations seen with reversible neuromuscular blockade, the most significant muscle fiber changes occurring subsequent to oculomotor nerve transection involved the orbital singly innervated fiber (type 1). At the light microscopic level, the principal morphopathological change was a progressive reduction in the coarse appearance of this fiber type (Fig. 1). Specifically, structural alterations, as seen with electron microscopy, consisted of dispersion of the central and subsarcolemmal mitochondrial aggregates that characterize these fibers (Fig. 6; see also Spencer and Porter11). Mitochondrial dispersion was initially detected at 7 days and was clearly evident at both light and electron microscopic levels by 28 days following oculomotor neuroectomy (Figs. 1B, 6B). Such alterations were first seen as dispersion of the central mitochondrial aggregates located in the immediate vicinity of neuromuscular junctions. Changes in mitochondrial content subsequently spread along the length of the fiber to extrajunctional zones and later involved the subsarcolemmal aggregates as well (Fig. 1C, 6C). Clear reductions in net mitochondrial content ultimately made this fiber type recognizable only by the persistence of AChE reaction product that allowed identification of its characteristic neuromuscular junction configuration (Fig. 6C). Mitochondrial dispersion was largely complete in 56-day survival cases, and few significant alterations occurred after this time.

Development of the extensive tubular aggregates which characterize alterations occurring in this fiber type subsequent to pharmacological neuromuscular blockade with botulinum toxin was not observed. Moreover, pathologies of the internal membrane system occurred only rarely in denervated orbital singly innervated fibers. In marked contrast to observations made for other skeletal muscles, the precise sarcomeric organization of myofilaments was largely intact for all survival times examined. Only a few muscle fibers showed any significant fragmentation of the Z-lines and disruption of the contractile apparatus (Fig. 6D). Vacuoles, possibly representing disintegrated mitochondria or lipid accumulations, were noted in some orbital singly-innervated fibers at longer (>112 days) survival times. Lysosomes sometimes were found in association with the peripheral mitochondrial aggregates present in this fiber type (Fig. 6C). The extensive microvascular network typically associated with this fiber type remained largely intact throughout the period of study.

Consistent with the qualitative evidence indicating only limited loss of myofilaments from denervated extraocular muscle, quantitative analysis showed slight hypertrophy in the orbital singly innervated fiber type at 28 days (cross-sectional area was 108% of control value), with a gradual drop to 87% and 54% of control value by 56 and 112 days, respectively (Fig. 5). Restoration of fiber cross-sectional area to values
Fig. 6. Electron photomicrographs of orbital singly innervated fibers from normal medial rectus muscle (A), and denervated muscle from 28- (B), 56- (C), and 167-day (D) survival cases. Central and subsarcolemmal mitochondrial aggregates (m) and the characteristic ring of capillaries (c) are seen in the normal fiber (A). An orbital multiply innervated fiber (MIF) is seen in the same micrograph. Orbital singly innervated fibers undergo progressive dispersion of mitochondrial aggregates, some fibers becoming vacuolated (v). In spite of disuse, myofibrillar components are largely intact. Persistence of AChE reaction product (filled arrows) and lysosomes (open arrows) are seen in (C). When present, myonuclei (mn) are indicated. \( \times 2800 \).

exceeding control (175% of control) occurred in the one 167-day experimental case with significant reinnervation.

The orbital layer multiply innervated fiber type (type 2 in Fig. 1) showed only slight structural alteration subsequent to oculomotor nerve transection. Occasional swelling of elements of the sarcoplasmic reticulum and limited myofilament loss was detectable. Mean fiber cross-sectional area exhibited changes much like those seen in the orbital singly
innervated fiber (Fig. 5). Following a modest hypertrophy by 28 days post-neurectomy (104% of control, \( P = 0.05 \)), this fiber underwent progressive atrophy (86% and 56% of control values at 56 and 112 days, respectively) followed by hypertrophy (123% of control) after significant reinnervation.

Global singly innervated fiber types are distinguished on the basis of content and distribution of mitochondria and degree of development of internal membrane systems.\(^1\)\(^2\)\(^3\) Since all exhibit the histochemical and ultrastructural profiles of fast-twitch muscle,\(^1\) these fibers were designated according to mitochondrial-associated myoglobin content (or color): global red singly innervated (type 3), global intermediate singly innervated (type 4), and global pale singly innervated fiber (type 5) types. The morphological profile of the global red singly innervated fiber most closely resembles that of the orbital singly innervated fiber, and structural alterations subsequent to denervation were similar in the two types (Fig. 1). Specifically, the type 3 fiber exhibited mitochondrial dispersion accompanied by only limited pathological change in the contractile apparatus. For short-term survival times (<56 days), type 4 and 5 fibers were affected even less, with most fibers exhibiting a virtually normal pattern of subcellular organelles (Fig. 7A). While a few globally singly innervated fibers showed abnormalities of internal membrane system elements, including focal proliferation and/or swelling of sarcoplasmic reticulum (Fig. 7C, D), the pattern of long-term changes resulting from oculomotor neurectomy was mild. Again, at long survival times, limited Z-line disruption and partial breakup of the myofibrillar organization were seen for some fibers (Fig. 7E, F), but such changes were rare. A few type 5 fibers exhibited loss of myofilaments from the subsarcolemmal region, similar to that seen in local anesthetic myotoxicity.\(^6\) Quantitative measures of the three global singly innervated fiber types revealed that, like the orbital layer fiber types, denervated extraocular muscles undergo a gradual and mild atrophy (Fig. 5). Mean fiber cross-sectional area was reduced only slightly at 28 days (92% of control). By 56 days, fiber area attained a low value of 73% of control and, with hypertrophy of a limited number of reinnervated fibers, rebounded to 85% of control value by 112 days. Like the orbital layer muscle fiber types, hypertrophy of global singly innervated fibers (156% of control) was seen following extensive reinnervation of the extraocular muscles.

On the basis of both qualitative and quantitative criteria, the global multiply innervated fiber type (type 6) was virtually unaffected by denervation (Figs. 5, 7B). Some fibers exhibited slight proliferation of internal membrane system components, but neither atrophy nor reinnervation-associated hypertrophy was seen for this fiber type.

**Regeneration and Reinnervation**

Longitudinal fiber splitting and rejection of fiber fragments (processes indicative of regeneration)\(^26\)\(^27\)\(^28\) were noted in the extraocular muscles after survival times of 56–112 days (Fig. 8A). Rejected muscle fiber fragments often appeared undifferentiated and were nearly devoid of contractile filaments. Concurrent with longitudinal fiber splitting was an apparent increase in number of satellite cells, a finding also suggestive of muscle regeneration. As proposed by Miledi and Slater,\(^29\) satellite cells may play an active role in longitudinal fiber splitting as their processes often intervened between associated muscle fiber fragments. Primitive neuromuscular junctions (Fig. 8B, C), consisting of small axon terminals applied to the surface of singly innervated fibers, began to appear after survival times of 56–112 days. At subsequent stages, these newly formed neuromuscular junctions exhibited larger axonal endings lying within depressions of the sarcolemma, much like those which characterize normal extraocular muscle. In the one case where the oculomotor nerve was cut cleanly and 167-day survival was allowed, significant reinnervation did occur although the characteristic layered organization of the extraocular muscles was retained and the fiber type grouping that typifies reinnervated skeletal muscle was not seen.

**Discussion**

While the pattern of denervation-induced changes in oculomotor axons and neuromuscular junctions is well documented,\(^30\)\(^31\) very little is known regarding the precise alterations that occur in the individual extraocular muscle fiber types. Moreover, those stud-
Fig. 8. Light and electron photomicrographs illustrating regenerative responses in denervated medial rectus muscles. Electron photomicrograph (A) shows a global singly innervated fiber undergoing rejection of an undifferentiated fragment (28-day survival). Inset: light photomicrograph of longitudinally split fiber from a 36-day survival case. Metachromatic staining of mast cell (mc) granules with toluidine blue also is seen. In low (B) and high (C) magnification electron photomicrographs, a neuromuscular junction is seen in association with a global intermediate singly innervated fiber (type 4; 167-day survival). When compared to the neuromuscular junctions normally observed for this fiber type, the primitive appearance of this junction suggests that it is a product of reinnervation. Preterminal axon (a), synaptic terminals (s), and typical postjunctional folding and extracellular AChE reaction product are seen overlying the myonucleus (mn). Contractile apparatus (mf) exhibits normal configuration. ×8000 (A, inset ×985), ×3750 (B), ×12,000 (C).
ies that have been concerned with fiber type-specific changes are difficult to interpret because of the perpetuation of a fiber classification scheme that is both incomplete and in error with regard to fiber type innervation pattern (singly innervated versus multiply innervated). By contrast, the current study relied on a comprehensive fiber classification scheme to distinguish both the general and fiber type-specific alterations that result from short- and long-term motor denervation of primate extraocular muscle. These results indicate that clear changes related to muscle fiber type, particularly in regard to mitochondrial content, occur in denervated extraocular muscles. Specifically, those singly innervated fibers with the highest mitochondrial content exhibited the most severe morphopathological changes. Moreover, observed changes in the extraocular muscle fiber contractile apparatus were surprisingly mild.

Dispersion and overall reduction in the mitochondria content of the orbital singly innervated fiber type and its global layer counterpart were the most conspicuous result of oculomotor neurectomy. On the basis of the histochemical and ultrastructural profile of this fiber type, it would exhibit fast-twitch and fatigue-resistant physiological properties, thereby suggesting that it is active over much of the oculomotor range. The observed mitochondrial alterations are consistent with the ensuing disuse that results from loss of motor innervation. A similar disuse-related mitochondrial change was seen in orbital singly innervated fibers of monkeys subjected to experimental neuromuscular blockade with botulinum toxin. While both interventions result in paralysis, either temporary or prolonged, the dispersion of central mitochondrial aggregates was characteristic of loss of neuromuscular transmission. The literature pertaining to extraocular muscle pathology presents an extended debate as to whether particular muscle changes are "neurogenic" or "myogenic" in origin. Data from this study, taken together with data from pharmacological paralysis of extraocular muscles, suggest that alterations in the distribution of mitochondria in the orbital singly innervated fiber may indeed be diagnostic of denervation.

Unlike botulinum-treated muscles, which develop extensive tubular aggregates in the orbital singly innervated fiber type, denervated muscles showed few changes in internal membrane system components. Since tubular aggregates appear to be adaptive proliferations of the sarcoplasmic reticulum related to altered calcium metabolism, their presence in botulinum paralysis, but not after motor nerve transection, may reflect a direct action of the toxin upon the muscle fiber proper.

The fundamental process occurring subsequent to elimination of motor innervation for most skeletal muscles at spinal levels is the rapid loss of contractile elements—the actin and myosin filaments. Myofilament breakdown is described as occurring in two stages: (1) a rapid phase, over the first 2–4 weeks following denervation, during which time fiber cross-sectional area is reduced by over 50%; and (2) a gradual filament disruption phase continuing until innervation is restored. Denervated extraocular muscles appear to bypass the first phase and undergo the later, limited, myofilament dispersion and reduction in fiber cross-sectional area. Although conflicting reports exist as to the quantitative changes that occur in denervated extraocular muscle, varying from no change to mixed changes, a pattern emerges that indicates that denervation atrophy is mild. While the precise explanation for the relative resistance to denervation exhibited by these muscles is not yet clear, perhaps the expression of unique myosin isoforms by adult extraocular muscles represents a "protective" factor. Extraocular muscle myosins may then be less dependent upon innervation than are those of other skeletal muscles. The issue of whether other musculature of the head region would exhibit similar behavior following denervation remains to be determined.

The infiltration of blood-borne immune system elements that is so characteristic of denervated skeletal muscle occurs in the extraocular muscles as well. While the extensive vascular network seen in primate extraocular muscles retracts following temporary denervation with botulinum toxin, this network is maintained following oculomotor nerve transection. Previous studies concerned with extraocular muscle denervation also report only slight alteration of the vasculature. Perhaps maintenance of the vascular network is necessary for the influx of immune system elements and subsequent removal of cellular debris. Mast cell infiltration is significant in that it represents a source of vasoactive substances (histamine and heparin, among others) to maintain the vasculature, but also mast cell granules are known to contain proteases that may assist in the removal of myelin following axon retraction.

Following denervation, oculomotor axon terminals retract and the characteristic triad of Schwann cell—axon terminal—muscle fiber is altered as the vacant synaptic space is occupied by a non-neural cellular element. Former sites of neuromuscular junctions were readily identifiable by persistence of AChE reaction product at these sites. Cells that overlie vacant synaptic sites have been designated interstitial cells and may be involved in synthesis of chemotactic factors. Recent studies suggest that specific...
modifications of muscle fiber cell surface and extracellular matrix may be instrumental in attracting new axons to denervated fibers.\textsuperscript{42-44} Normal muscle fibers exhibit neural cell adhesion molecule (NCAM) only in association with the neuromuscular junction. Following denervation, NCAM and other glycoproteins are reexpressed and then are found in nonjunctional regions of the sarcolemma and in the extracellular matrix in the vicinity of former junctions. Since ingrowing axons preferentially target former synaptic sites,\textsuperscript{45,46} NCAM, and possibly other factors such as persistent junctional AChE, may serve to attract neuronal growth cones.

The potential neuronal source for the limited reinnervation of denervated extraocular muscles remains undetermined. The manner in which nerve transaction was performed in the present study allowed the survival of only few oculomotor motoneurons. The reinnervation that was noted may then be derived from these few surviving neurons or, alternatively, collateral sprouting of abducens or trochlear motoneurons. Although both forms of reinnervation have been demonstrated for the extraocular muscles,\textsuperscript{47-49} oculomotor motoneurons do not tolerate central axotomy (current results; Baker et al\textsuperscript{47}) and thus are an unlikely source for muscle reinnervation.

In one experimental case, significant reinnervation was noted. In contrast to observations in other skeletal musculature, fiber type grouping (ie, the presence of tight clusters of particular fiber types) was not observed. These data suggest that extraocular muscle fibers may selectively attract axons of motoneurons with the “correct” physiological properties such that individual fiber characteristics are not respecified. Alternatively, the fiber types present in these muscles may not be as susceptible to change by alterations in neuronal discharge pattern. Instead, reinnervation may simply allow restoration of those characteristics that were originally expressed by a particular fiber.

While the concept of recruitment of successively stronger, faster and more fatigable motor units in order to smoothly increment tension during movement is well established for other skeletal muscles,\textsuperscript{50} such considerations have been largely ignored in the oculomotor system. With the delineation of distinct fiber types in mammalian extraocular muscles and data suggesting a differential response of these types to experimental manipulation, it is clear that the lack of knowledge of motor unit properties represents a major gap in our understanding of the oculomotor system. Scott and Collins\textsuperscript{8} proposed a division of labor among extraocular muscle fiber types on the basis of type and amount of work performed, and not, as was proposed earlier,\textsuperscript{51,52} on the basis of their relative contributions to different eye movement subsystems. While it is no longer tenable that particular extraocular muscle fiber types may subserve vergence movements while others function only in saccades, the presence of fiber types with different contraction speeds and degrees of fatigability may provide the basis for progressive augmentation of tension. The presence of large mitochondrial aggregates in the orbital singly innervated fiber is suggestive of oxidative capacity that would place it among the most fatigue-resistant fiber types found in mammalian muscles. The morphological profile of this fiber type also is consistent with the extremely high sustained discharge rates exhibited by oculomotor motoneurons.\textsuperscript{53-55} On the basis of the histochemical and ultrastructural characteristics of the orbital singly innervated muscle fiber, Spencer and coworkers\textsuperscript{5,56} have postulated that this fiber type is recruited very early in eye movements and remains active in maintaining fixation over much of the oculomotor range. Electromyographic data, which indicate that the orbital layer fibers provide continuous force when the eye is in primary position and slowly increment tension with movements in the on-direction of the muscle, lend credence to this idea.\textsuperscript{8} Present data, indicating that the orbital singly innervated fiber exhibits the most conspicuous alterations following denervation, lend support for the projected role of this fiber in eye movements.

Key words: extraocular muscle, oculomotor, denervation, eye movement, cynomolgous monkey, \textit{Macaca fascicularis}

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